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ABSTRACT

Intended to be used along with the "Teacher's Guide to Classroom Discussions for Biology" and the "Teacher's Guide to Laboratory Activities for Biology," this volume presents 43 laboratory exercises for introductory college-level biology. (CP)

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# LABORATORY ACTIVITIES FOR BIOLOGY

## Thirteen Colleges Curriculum Program
## The Five College Consortium
## The Eight College Consortium

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**Second Edition**

C. M. GOOLSBY

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Second Printing, January, 1972

INSTITUTE FOR SERVICES TO EDUCATION, INC.

# LABORATORY ACTIVITIES FOR BIOLOGY

## Thirteen Colleges Curriculum Program

## The Five College Consortium

## The Eight College Consortium

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Institute for Services to Education
Washington, D.C.

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Northeastern University
Boston, Massachusetts

1971

## PREFACE TO THE SECOND EDITION

*Laboratory Activities for Biology* has been tested by some 1,500 students during the 1970-1971 school year, and was very successfully used by teachers in the Thirteen College Curriculum Program and in the Five College Consortium. This edition reflects the changes that teachers felt would improve the usefulness of this workbook in guiding students through laboratory experiences in the biology course. Teachers use this manual together with the new *Teacher's Guide To Laboratory Activities for Biology* and *Teacher's Guide to Classroom Discussions for Biology*, both being publications of the Institute for Services to Education.


## ACKNOWLEDGEMENTS

We gratefully acknowledge the contributions of the following people to this compilation. Dr. Paris M. Allen of the ISE staff wrote Exercise 6. Part of Exercise 16 was contributed by Reid Jackson of the Harvard Graduate School of Education. Martin J. Carey of Clark College developed parts of Exercise 30, and Robert J. Anthony of Jackson State College wrote Exercise 42.


Charles M. Goolsby
Washington, D. C.
May, 1971

## PREFACE TO THE FIRST EDITION

The Thirteen Colleges Curriculum Program was started in 1967 as a cooperative venture in curriculum development between the colleges and the Institute for Services to Education through its Curriculum Resources Group. When the Biology course became defined it sought to provide laboratory experiences which were relevant to students and which captured their interests and curiosity. By the summer of 1969 teachers in the Biology program were able to indicate concretely the kind of laboratory experiments which were of most interest. The present series of laboratory activities is based upon the kinds of exercises and experiments that they indicated as being appropriate and interesting to the beginning college student. Because the experiments and exercises were appended to eight separate units of study there was some overlap of experiments and of units to which they were related. In many cases related exercises and experiments were brought together as parts of a single exercise. A few new exercises have been added. In the Table of Contents the units of the 1969 CRG Biology Teacher's Curriculum Guide to which the exercise is related has been indicated.

Charles M. Goolsby
Newton, Massachusetts
July, 1970

The biology teachers who have participated in this development through the summer of 1970 are:

ALABAMA A & M COLLEGE: **Jimmie L. Cal**, M.Ed. (1967-1970), **Rather Brown**, M.S. (1970- ), **George Grayson**, M.S. (1970S)

BENNETT COLLEGE: **Perry V. Mack**, M.S. (1967- )

BISHOP COLLEGE: **Willie M. Clark**, M.S. (1967-1970), **Mrs. Versia Lindsay Lacy**, M.S. (1970- ), In the Modified-ISE course: **Wasi M. Siddiqui**, Ph.D. (1969- ), **Herbert Alexander**, M.S. (1969- ), **Ehsan A. Syed**, M.S. (1969- ), **Mrs. Rose W. Burke**, M.A. (1969-1970)

CLARK COLLEGE: **Martin J. Carey**, M.S. (1967- ), **F. Rusinko**, M.S. (1970S)

FLORIDA A & M UNIVERSITY: **Louis Stallworth**, M.S. (1967-1969), **Purcell B. Bowser**, M.S. (1969-1970), **Mrs. Irene R. Clark**, M.Ed. (1970- )

JACKSON STATE COLLEGE: **Robert J. Anthony**, M.S., M.Ed. (1967- ), **Mrs. B. Henderson**, M.S. (1970S)

LINCOLN UNIVERSITY: **Harold C. Banks**, M.S. (1967-1968, 1969- )

NORFOLK STATE COLLEGE: **Ruth E. Churwin**, M.A. (1967-1969), **Mrs. Irene R. Clark**, M.Ed. (1969-1970), **Mrs. Robin M. Griffith**, M.A., M.A.T. (1970- )

NORTH CAROLINA A & T STATE UNIVERSITY: **Mrs. Elizabeth D. Clark**, M.S. (1967- )

SOUTHERN UNIVERSITY, Baton Rouge, Louisiana: **Robert H. Cobbins**, M.S. (1967- )

TALLADEGA COLLEGE: **Muriel E. Taylor**, M.A. (1967- ), **Mrs. Mae T. Groves**, M.S. (1970- )

TENNESSEE STATE UNIVERSITY: **Mrs. Alice C. Smith**, M.S. (1967- )

Beginning with the summer of 1970 teachers from six additional institutions are participating in the curriculum development program. They are:

ELIZABETH CITY STATE UNIVERSITY: **Thaddeus V. Beasley**, M.S.

FAYETTEVILLE STATE UNIVERSITY: **Mrs. Valerie L. Fleming**, M.S.

LANGSTON UNIVERSITY: **Harold W. Toliver**, M.S.

SAINT AUGUSTINE'S COLLEGE: **Chandra P. Misra**, Ph.D.

SOUTHERN UNIVERSITY, Shreveport, Louisiana: **Mrs. Rebecca B. Anderson**, M.S.T.

TEXAS SOUTHERN UNIVERSITY: **Charles H. Bennett**, M.S.

Program Associates from the INSTITUTE FOR SERVICES TO EDUCATION:
**Charles M. Goolsby**, Ph.D. (1968- ), **Dan A. Obasun**, Ph.D. (1969- )
**Paris M. Allen**, Ph.D. (1969-1970)

## ABOUT THE INSTITUTE FOR SERVICES TO EDUCATION

The Institute for Services to Education was incorporated as a non-profit organization in 1965 and received a basic grant from the Carnegie Corporation of New York. The organization is founded on the principle that education today requires a fresh examination of what is worth teaching and how to teach it. ISE undertakes a variety of educational tasks, working cooperatively with other educational institutions, under grants from government agencies and private foundations. ISE is a catalyst for change. It does not just produce educational materials or techniques that are innovative; it develops, in cooperation with teachers and administrators, procedures for effective installation of successful materials and techniques in the colleges.

ISE is headed by Dr. Elias Blake, Jr., a former teacher and is staffed by college teachers with experience in working with disadvantaged youth and Black youth in educational settings both in predominantly Black and predominantly white colleges and schools.

ISE's Board of Directors consists of persons in the higher education system with histories of involvement in curriculum change. The Board members are:

| | |
|---|---|
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## ABOUT THE THIRTEEN-COLLEGE CURRICULUM PROGRAM

From 1967 to the present, ISE has been working cooperatively with the Thirteen-College Consortium in developing the Thirteen-College Curriculum Program. The Thirteen-College Curriculum Program is an educational experiment that includes developing new curricular materials for the entire freshman year of college in the areas of English, mathematics, social science, physical science, and biology and two sophomore year courses, humanities and philosophy. The program is designed to reduce the attrition rate of entering freshmen through well thought-out, new curricular materials, new teaching styles, and new faculty arrangements for instruction. In addition, the program seeks to alter the educational pattern of the institutions involved by changing blocks of courses rather than by developing single courses. In this sense, the Thirteen-College Curriculum Program is viewed not only as a curriculum program with a consistent set of academic goals for the separate courses, but also as a vehicle to produce new and pertinent educational changes within the consortium institutions. At ISE, the program is directed by Dr. Frederick S. Humphries,

Vice-President. The curricular developments for the specific courses and evaluation of the program are provided by the following persons:

| Course | ISE STAFF |
|---|---|
| English | Miss Joan Murrell, Senior Program Associate<br>Miss Carolyn Fitchett, Program Associate<br>Mr. Sloan Williams, Program Associate<br>Miss Tiveeda Williams, Research Assistant<br>Miss Ernestine Brown, Secretary |
| Social Science | Dr. George King, Senior Program Associate<br>Dr. William J. Massie, Jr., Program Associate<br>Miss Nancy Seymour, Research Assistant<br>Miss Gloria Lowe, Secretary |
| Mathematics | Dr. Beauregard Stubblefield, Senior Program Associate<br>Mr. Bernis Barnes, Program Associate<br>Dr. Phillip McNeil, Program Associate<br>Dr. Walter Talbot, Consultant<br>Mrs. Debrah Johnson, Secretary |
| Physical Science | Dr. Leroy Colquitt, Senior Program Associate<br>Dr. Ralph Turner, Consultant<br>Mrs. Cynthia Paige, Secretary |
| Biology | Dr. Charles Goolsby, Senior Program Associate<br>Dr. Daniel Obasun, Program Associate<br>Dr. Paul Brown, Consultant |
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| Philosophy | Dr. Conrad Snowden, Senior Program Associate<br>Dr. Henry Olela, Program Associate<br>Miss Valerie Simms, Program Associate<br>Miss Judith Burke, Research Assistant<br>Miss Faith Halper, Secretary |
| Evaluation | Dr. Thomas Parmeter, Senior Research Associate<br>Dr. Joseph Turner, Senior Research Associate<br>Mr. John Faxio, Research Assistant<br>Mrs. Judith Rogers, Secretary |

In addition, Miss Patricia Parrish serves as general editor of the curriculum materials as well as an Administrative Assistant to the Director. Mrs. Joan Cooke is Secretary to the Director.

The curriculum staff is assisted in the generation of new educational ideas and teaching strategies by teachers in the participating colleges and outside consultants. Each of the curriculum areas has its own advisory committee, with members drawn from distinguished scholars in the field but outside the program.

The number of colleges participating in the program has grown from the original thirteen of 1967 to nineteen in 1970. The original thirteen colleges are:

| | |
|---|---|
| Alabama A and M University | Huntsville, Alabama |
| Bennett College | Greensboro, North Carolina |
| Bishop College | Dallas, Texas |
| Clark College | Atlanta, Georgia |
| Florida A and M University | Tallahassee, Florida |
| Jackson State College | Jackson, Mississippi |
| Lincoln University | Lincoln University, Pennsylvania |
| Norfolk State College | Norfolk, Virginia |
| North Carolina A and T State University | Greensboro, North Carolina |
| Southern University | Baton Rouge, Louisiana |
| Talladega College | Talladega, Alabama |
| Tennessee State University | Nashville, Tennessee |
| Voorhees College | Denmark, South Carolina |

A fourteenth college joined this consortium in 1968, although it is still called the Thirteen-College Consortium. The fourteenth member is:

| | |
|---|---|
| Mary Holmes Junior College | West Point, Mississippi |

In 1970, five more colleges joined the effort although linking up as a separate consortium. The members of the Five-College Consortium are:

| | |
|---|---|
| Elizabeth City State University | Elizabeth City, North Carolina |
| Langston University | Langston, Oklahoma |
| Southern University at Shreveport | Shreveport, Louisiana |
| Saint Augustine's College | Raleigh, North Carolina |
| Texas Southern University | Houston, Texas |

In 1971, eight more colleges joined the curriculum development effort as another consortium. The member schools of the Eight College Consortium are:

| | |
|---|---|
| Alcorn A and M College | Lorman, Mississippi |
| Bethune-Cookman College | Daytona Beach, Florida |
| Grambling College | Grambling, Louisiana |
| Jarvis Christian College | Hawkins, Texas |
| LeMoyne-Owen College | Memphis, Tennessee |
| Southern University in New Orleans | New Orleans, Louisiana |
| University of Maryland, Eastern Shore | Princess Anne, Maryland |
| Virginia Union University | Richmond, Virginia |

The Thirteen-College Curriculum Program has been supported by grants from:

The Office of Education, Title III, Division of College Support
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The National Science Foundation, Division of the Undergraduate Education
The Ford Foundation
The Carnegie Corporation
The Esso Foundation

# TABLE OF CONTENTS


| EXERCISE | RELATED UNITS | TITLE |
|---|---|---|
| 1 | 1 | WHAT IS AN EXPERIMENT? SCIENTIFIC REPORTS |
| 2 | 1 | WEIGHING AND MEASURING |
| 3 | 1, 2 | DIFFRACTION OF LIGHT INTO ITS SPECTRUM |
| 4 | 1, 2 | THE MICROSCOPE AND MICROSCOPY |
| 5 | 2, 3 | STERILIZING AND STERILE TECHNIQUE |
| 6 | 2, 5 | EFFECT OF ULTRAVIOLET LIGHT RADIATION ON THE COLOR OF *SERRATIA MARCESCENS* |
| 7 | 2, 3 | BUFFERS AND INDICATORS |
| 8 | 2, 3 | COACERVATES AND EMULSIONS |
| 9 | 2, 3 | WATER CONTENT OF VARIOUS TISSUES AND CELLS |
| 10 | 2, 3, 7 | CELL TYPES |
| 11 | 3, 6 | TYPES OF FOODS FOUND IN CELLS AND TISSUES |
| 12 | 3 | DIFFUSION, OSMOSIS AND ACTIVE TRANSPORT |
| 13 | 3, 6 | ACTIVITIES OF ENZYMES |
| 14 | 3, 6 | FERMENTATION AND AEROBIC RESPIRATION COMPARED |
| 15 | 3, 5 | CHROMOSOME MOVEMENTS DURING CELL DIVISION |
| 16 | 3, 5 | CELL DIVISION |
| 17 | 7 | THE PHYLA OF THE ANIMAL AND PLANT KINGDOMS |
| 18 | 2, 6, 7 | COMPARATIVE ANATOMY OF SOME SKELETAL FEATURES |
| 19 | 4, 6, 7 | DISSECTION OF THE FETAL PIG |
| 20 | 4, 7 | SEXUAL REPRODUCTION AND VEGETATIVE REPRODUCTION (WITHOUT RESPECT TO SEX) |
| 21 | 4, 7 | REPRODUCTIVE STRUCTURES IN FLOWERING PLANTS (ANGIOSPERMS) |
| 22 | 4, 7 | SEED AND FRUIT PRODUCTION IN FLOWERING PLANTS |
| 23 | 7 | MONOCOT AND DICOT SEEDS, SEEDLINGS AND LEAVES |
| 24 | 4 | HISTOLOGY OF THE FEMALE REPRODUCTIVE TRACT |
| 25 | 4 | HISTOLOGY OF THE MALE REPRODUCTIVE TRACT |
| 26 | 4, 6 | RESPONSE OF ANIMALS TO PREGNANCY URINE HORMONE |
| 27 | 3, 5 | TRANSCRIBING DNA, mRNA AND tRNA TO SEQUENCE A PROTEIN |
| 28 | 2, 5 | DO ENVIRONMENTAL FACTORS AFFECT THE ACTION OF GENES? |
| 29 | 5 | THE INHERITANCE OF GENETIC TRAITS |
| 30 | 5, 6 | SEARCHING FOR BACTERIA RESISTANT TO STREPTOMYCIN AND INCREASED SALT CONCENTRATIONS |

## TABLE OF CONTENTS (continued)


| EXERCISE | RELATED UNITS | TITLE |
|---|---|---|
| 31 | 2, 3, 5 | INHERITANCE OF SPECIFIC PROTEINS |
| 32 | 6 | PHOTOSYNTHESIS AND THE SYNTHESIS OF STARCH |
| 33 | 6 | DIGESTION OF FOODS |
| 34 | 6 | MEASUREMENT OF OXYGEN USE |
| 35 | 6 | URINALYSIS (URINE ANALYSIS) |
| 36 | 6 | REFLEX ACTION |
| 37 | 4, 6, 7 | INFLUENCE OF THYROID HORMONE ON RATE OF DEVELOPMENT |
| 38 | 8 | A STUDY OF PLANT COMMUNITIES |
| 39 | 8 | SUCCESSION FROM ONE ENVIRONMENT TO ANOTHER |
| 40 | 8 | EFFECT OF PHYSICAL AND CHEMICAL FACTORS ON ANIMALS |
| 41 | 8 | TERMITE–FLAGELLATE INTERACTION |
| 42 | 8 | GROWTH OF A POPULATION |

## EXERCISE 1 – SCIENTIFIC METHOD

The "scientific method" as used in biology is a testing procedure which has come to be accepted by scientists. It has reached its present state after men of science in different centuries have found that its several components were necessary in order to take concepts and philosophies and use them to solve both practical and theoretical problems. Therefore, a scientific experiemnt is an experience in applied philosophy.

Because it took a long time for men to discover this acceptable way of thinking about natural occurrences, of investigating questions that made them curious, and of reasoning about the results of their investigations, we will not depend upon discovery for you to find out what the steps were. Rather we shall outline them and then let you discover how it works in a real experiment.

Before we begin let us distinguish between experiments and exercises. All of the activities in this manual are headed "Exercise ." This is a convention for teaching convenience. An exercise is an activity where you acquire a skill that you may need to use later. An experiment is a procedure for finding out information. We can tell you what the skill is that you should learn in an exercise, but we cannot always tell you exactly the outcome of the experiment.

Steps In The Scientific Method

1. *Observation* of a phenomenon in the field or in the laboratory which stirs the curiosity of the observer.

2. Formation of an *hypothesis* (or probable cause) for the phenomenon. This may be an "educated guess" or maybe even a plain guess. It usually is expressed as a question or as a statement to be proved right or wrong.

3. *Design of an experiment* that will test the hypothesis. There are several ways to sequence an experiment but it must have one or more *control* groups. The Control group represents the "normal" or what would happen if the test object was not treated in any unusual way, as a rule. Something will be changed for the *experimental* groups. A drug will be given, another chemical used, the temperature changed, or the acidity will be different. Sometimes experimental subjects (such as a human patient, or a student) may not be available except one at a time. In such cases the same individual may be the experimental and the control for the experiment.

For example, a small child has an eczema (rash), intense itching, and shows signs of headache after certain meals, particularly lunch. Peanut butter and chocolate are often served for that meal and so they are suspected to be the cause. That suspicion constitutes an hypothesis. Both chocolate and peanut butter are withheld from all meals for a week, during which time the rash, itching and headache gradually disappear. The child is in the normal condition again and so this is the control, at the end of the week.

Chocolate is then allowed for lunch and the itching returns in minutes. It will not be given the child again. After a few days when he has returned to normal he is permitted a peanut butter sandwich (which he likes very much). There are none of the undesirable reactions. On the basis of these observations one can come to only one position regarding the child's sensitivity toward chocolate and peanut butter. The experiment is over and one subject has served as both normal control and abnormal experimental subject.

A more usual type of experiment used in the laboratory is one that makes use of many experimental subjects (animals, plants, tube tests) so that normal control reactions can be determined at the same time as one or many expermental groups are being tested. This is one way to save a great deal of time.

4. *Collection and Analysis of Data.* Data may be observational (or qualitative) such as the color of a leaf or of a test tube test; or it may be measurement data (that is size, temperature, weight, etc.).

5. *Reaching conclusions* based on the data. Sometimes an experiment may be done to demonstrate a law for the class. In this case one tries to explain the results by saying, "The X-Law says that this must happen, and you see that it did." Deduction, however, has not led to much scientific discovery. The usual procedure is to reason from the data (facts) toward an expression which summarizes the activity. Such a conclusion is called a generalization and this process of reasoning from the particular (facts) to the general (statement) is called reasoning by *induction* in contrast with reasoning by deduction.

6. *Acceptance or rejection of the starting hypothesis.*

7. Formulation of a continuation hypothesis to explain unexplained questions arising from the experiment.

Here is a simple experiment. After you have done it review the steps above and see what parts of the activity correspond with the steps in the scientific method.

"The Black Box" or "The Christmas Present" Experiment
(Suggested by Willie M. Clark, Bishop College)

You will be provided with a closed box of small size containing an object. Your task is to determine as many facts about the object in the box as you can and to finally guess what is in the box. A set of objects, some, all or none of which may be in boxes given to members of the class are available. There will also be some balances and perhaps an empty box like yours. Write down your observations as you go along and also your conclusion as to the contents. The teacher will let you know when you can open the box. Were you right? If you were wrong, how do you feel about it? If you were wrong, obtain a second box and see if you can do any better. Relate what you did to the steps in the scientific method.

## SCIENTIFIC REPORTS (ORIGINAL PAPERS)[1]

Scientific reports in biology reflect the use of the scientific method. They are usually written in five sections and scholars and researchers have come to look in specific sections for the kind of information they seek.

The first section is the INTRODUCTION which gives the historical precedents for doing the experiment or experiments. The introduction ends with the *hypotheses* or *objectives* of the experiments. It then follows that the historical part should marshall the evidence that the objectives are reasonable.

The second section is called MATERIALS AND METHODS as a general rule. In this section are listed the materials used, specific organs or tissues used, the organism from which they came, and the procedure used. (This section may come from the laboratory instructions so that they may be included as they are, but with any additional items properly entered.)

The RESULTS section is third. In this section report your laboratory data and the requested computations (as a minimum) based thereon. In order to have results to report, it will be necessary to keep a laboratory notebook. The *notebook* is required of all students. Complete your data and computations in your notebook.

The REPORT SHEETS for the exercises in this workbook constitute a kind of notebook.

[1] This section is written as direction to the student for writing up a scientific report of his own. It contains, however, the same basic directions followed by the writers of most scientific reports in biology and biochemistry.

The DISCUSSION is the name of the fourth section. The fundamental aim here is to *explain* what happened in the experiment. This process of explanation will involve *looking up information* so that you can tell whether or not your results are in line with the findings of others. The chief source of this information is to be found in original papers appearing in the scientific journals, reference treatises and sometimes even in textbooks and manuals. The best way to begin looking up such information is to go to an abstract journal such as *Chemical Abstracts* or *Biological Abstracts*. Look up the subject in the annual indices, starting with the most recent and working backward through the years. Index references in Chemical Abstracts are by column number followed by a location letter. Starting with Vol. 66, Chemical Abstracts are listed by number. Biological Abstracts are listed by abstract number. In both cases abstracts are grouped by a category, but many times they may be in some other category also. Because of this, the abstract will be cross-referenced by several subjects, animals, organs or procedures. The abstract is an abbreviated summary of an original article. By reading it you may either obtain the desired information or you may determine whether or not you ought to read the original paper. The standard form for references is given in the next section. Other primary sources are the Annual Review of Physiology, Annual Review of Biochemistry, and others in the Annual Review series. Advances in Carbohydrate Chemistry, Advances in Colloid Chemistry, Recent Advances in Hormone Research are titles of other annual series which seek to review recent developments. A list of journals relevant to this course will be found on the laboratory bulletin board.

The fifth section of the report is entitled SUMMARY AND CONCLUSIONS. This is a short section which closes the report. Note that this section has two parts. The summary precedes the conclusions based on *your* data.

The last part of the report is a list of REFERENCES used. The term "Bibliography" is used only for *complete* listings of books and papers on a given subject and therefore will not apply here. References may be made several ways in the text of the report. We shall consider it standard in this course to list the names of the author or authors in the same order in which they appear under the title of the original paper, followed by the year of publication (in parentheses). If the same sequence of authors has more than one publication add a small a, b, c, etc. after the year. In the reference list give the last name of the first author followed by his initials. If there is more than one author, those after the first are listed initials first. After this comes the year of publication, then the title of the paper, the journal, the volume and page numbers.

Examples:

Clausen, H. H. 1940. The atrophy of the adrenal cortex following the administration of large amounts of progesterone. Endocrinology, 27:989–993.

Gaunt, R. G., C. H. Tuthill, N. Antonchak, J. H. Leathem 1953. Antagonists to cortisone. An ACTH-like action of steroids. Endocrinology, 54:272–283.

Here are two examples of book references:

Fieser, L. F. and M. Fieser 1949. *Natural Products Related to Phenanthrenes* American Chemical Society monograph, Rheinhold Publishing Co., New York. 704 pp. Page 146.

Dorfman, R. I. 1948. *Biochemistry of Androgens*, in G. Pincus and K. V. Thimann (editors), The Hormones I, Academic Press, Inc., N.Y. 886 pp. Pages 467–548.

## REPORT SHEET FOR EXERCISE 1

Notes on the Black Box (Christmas Present) Experiment

What do you think is in the box (before you open it)?

Were you right?
How do you feel about it?
Do you think that you should replace the object with one you thought should have been there if you were wrong?
Are you going to try the experiment again with another unknown box?
If so, use another sheet of paper for your notes and include it here.

## EXERCISE 2 – CONCEPTS OF MASS WEIGHT AND DENSITY

### Introduction

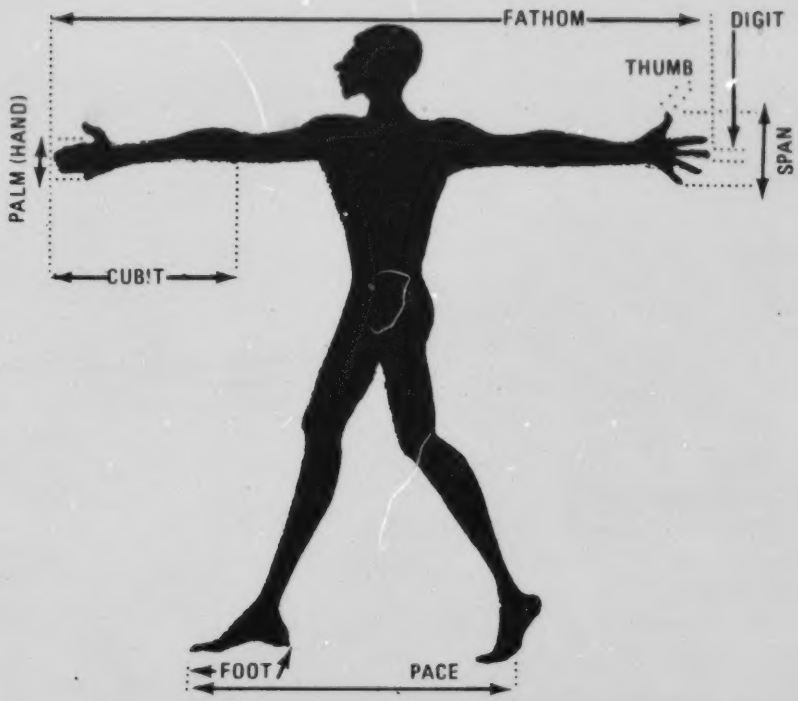
The common unit of weight in the scientific laboratory is the gram. This unit may be multiplied to give heavier units for convenience in weighing heavy objects or subdivided into smaller units for greater accuracy in weighing light objects, especially small objects. Prefixes used to designate multiples or fractions of a standard mass (weight), distance, quantity, etc. are as follows:

| Prefix | Meaning | Numerical Value | Symbol |
|---|---|---|---|
| Mega – | One Million | $10^{6}$ or 1,000,000 | M |
| Kilo – | One Thousand | $10^{3}$ or 1,000 | K |
| Deci – | One Tenth | $10^{-1}$ or .10 | d |
| Centi – | One Hundredth | $10^{-2}$ or .01 | c |
| Milli – | One Thousandth | $10^{-3}$ or .001 | m |
| Micro – | One Millionth | $10^{-6}$ or .000001 | $\mu$ |
| Nano – | One Billionth | $10^{-9}$ or .000000001 | n |

Therefore, multiples or fractions of a gram would be indicated by adding the appropriate prefix to the term gram, viz: Kilogram, milligram, or microgram.

| Unit of Weight | Comparative Weight | Abbreviation or Symbol |
|---|---|---|
| Kilogram | 1000 Gm. | Kgm. |
| Centigram | 100 Gm. | Cgm. |
| Gram | 1 Gm. | Gm. |
| Milligram | .001 Gm. | mg. or mgm. |
| Microgram | .001 Mg. | $\mu$g. or $\gamma$ (gamma) |

| Unit of Measure | Comparative Length | Abbreviation or Symbol |
|---|---|---|
| Kilometer | 1000 M. | Km. |
| Meter | 1 M. | M. |
| Centimeter | .01 M. | cm. |
| Millimeter | .1 cm. | mm. |
| Micron | .001 mm. | $\mu$ or mu |
| Millimicron | .001 micron | m$\mu$ |
| Nanometer | .001 micron | nM |
| Ångstrom unit | .1 nM | Å |




Drawing by Dorothy D'Anna

**Man was the original measuring tool. At one time or another, almost every part of the human body was used to measure length. But since one man's foot might be twice the length of his neighbor's, uniform lengths had to be established. And eventually standard feet, yards, etc. were marked on rods and bars to serve as units of length.**

The metric system got its name from the meter (meaning measure) and was conceived in France in 1792. The scientists that formulated it based it upon what they thought were "natural standards." The unit of length, the meter, was supposedly 1/10,000,000th of the distance from the equator to the North Pole on a line passing through Paris. This distance was represented by a platinum alloy cylinder kept in Paris. In 1960 the meter was re-defined as 1,650,763.73 wavelengths of the reddish-orange light emitted by a lamp with a krypton-86 ($Kr^{86}$) filament at a temperature of -210° Celsius (formerly Centigrade). The meter, therefore, is not 1/10 millionth the distance from the Pole to the Equator but so many wavelengths of an arbitrarily selected color of light.

A gram was defined as the weight of 1 cubic centimeter of water at 4° Celsius. However, 1 gram is small, only 0.0353 or just under 1/28 ounce, so that the kilogram was declared the international standard mass. A cylinder of platinum weighing just as much as 1 cubic decimeter (10 cm. per side) of water at 4.0°C., was the original standard and was known as the Kilogram of the Archives. It also was kept near Paris. This was replaced with a harder platinum-iridium alloy cylinder in 1889 and is maintained near Paris as Prototype Kilogram No. 1. As nearly exact duplicates as it is possible for our engineering to make have been prepared and sent to different countries. Prototype Kilogram No. 20 is kept in the National Bureau of Standards in Washington, D.C., and all working weights in the United States are directly or indirectly compared with it. Thus a kilogram is no longer the weight of 1000 cubic centimeters of pure water but the weight of a standard piece of metal.

**Useful Formulae**

Area of a
- square or rectangle = length x width
- triangle = ½ base x height
- trapezoid = base x altitude
- circle = $\pi r^2 = \frac{1}{4}\pi d^2$ ($\pi = 3.1416$)
- sphere = $4\ \pi r^2$

Volume of a
- cube or rectangular block = area of base x height
- prism = area of base x altitude
- cylinder = area of base x altitude
- cone or pyramid = 1/3 area of base x altitude
- sphere = $4/3\ \pi r^2$

**Materials and Equipment**

1 triple beam balance
1 aluminum weighing pan
1 Physiological weight (5 or 10 gms.)
1 10-ml. graduated cylinder
1 metric ruler (cm. and mm.)
1 10-ml. pipette
1 1-ml. pipette
1 large nail
Ethyl alcohol
Glycerol
4 small beakers

**Part A. Weight of an Aluminum Pan and of a Physiological Weight**

Be sure that the balance is level and that the weights are at ZERO on their scales.
Place the aluminum weighing pan on the balance.
Adjust the weights until the beam swings equidistantly above and below the index mark for the beam end.
Record the weight of the pan (tare weight).
Now add the physiological weight to the weighing pan.
Adjust the weights. Read and record the gross weight.
Return the weights to ZERO and compute the net weight of the bar.

**Part B. Measuring A Solid**

1. Determining the volume of an object of easily measured dimensions:
   Take a mm. ruler and measure the physiological weight, estimating the fractions of a mm. along each edge (length, width, depth).
   Record each measurement and compute the volume.
   List on your answer sheet any factors which affect the accuracy of the measurement of this item.

   Take a 10 ml. pipette.
   Draw water up into it above the 5 ml. mark.
   Quickly place the index finger over the top of the pipette.
   Release fluid from the pipette until the base of the meniscus is exactly on the 5 ml. mark.

   Now transfer the water to a 10 ml. graduated cylinder. If the pipette has a ground glass ring at the mouthpiece you must blow out the last drop of water for accurate measurement. Some pipettes, called measuring pipettes, are not graduated to the end of the tip. *Always check the kind of pipette you are using.* The fluid in the cylinder should come exactly to the 5.0 ml. mark. If it doesn't, adjust it with a dropper and continue to

practice the art of filling and emptying your pipette until you have mastered the technique with accuracy.

Place the physiological weight into the graduated cylinder by letting it slide gently down the side of the cylinder.
Read the volume at the base of the meniscus. Record it.
Subtract the original volume (5.0 ml.) from the final volume.
How does this compare with the volume determined by measuring with a ruler?

Now weigh a hard to measure object like a large nail and determine its volume using the graduated cylinder.
What is the difference between scales and a balance?
Is gravity the same strength at all points on the surface of the earth?

2. Density

Divide the weight of the physiological weight by its volume to determine the grams or milligrams per cubic centimeter.
Now divide the volume by the weight to determine the cubic mm. per mg. of weight.
Convert these values to grams per cubic centimeter.
What is the weight per unit of volume called?

3. Pipetting and Viscosity

Fill the pipette exactly to the 1.0 ml. mark with water. Determine the time in seconds that it takes for the water to run out. Repeat this four more times. Average your results and determine the standard error of the mean. (See page 9-1 for procedure.)

Using the same pipette, repeat the above procedure using alcohol, then do it with glycerol. Before starting the trials with a new solution, rinse out the pipette with some of the next solution so that you will measure the behavior of a homogeneous mixture. Repeatability depends upon the accuracy with which you fill the pipette.

Using the time that it took water to run out of the pipette as 1.000, compute what the relative viscosities of alcohol and glycerol would be by dividing the time for water into the other times.

## REPORT SHEET FOR EXERCISE 2

### Part A. Weights

Gross weight (pan and physiol. weight) _____ gms. or _____ mgms.
Tare weight (pan alone) _____ gms. or _____ mgms.
Net weight of physiological weight _____ gms. or _____ mgms.

Weight of the large nail _____ gms. or _____ mgms.

### Part B. Measuring a Solid

Physiological weight: Length _____ mm, Width _____ mm, Depth _____ mm.

Volume = _____ $mm^3$ or _____ $cm^3$.

Factors which made the most accurate measurement uncertain were:

_____

_____

_____

Volume measured in a cylinder:

Volume after adding the weight _____ ml.
Original volume of water _____ ml.
Volume of solid _____ ml. or _____ $mm^3$.

Volume after adding the nail _____ ml.
Original volume of water _____ ml.
Volume of solid _____ ml. or _____ $mm^3$.

Factors affecting the accuracy of the measurement of volumes by this method include:

2. Density

Physiological weight: d = Wt./vol. = _____ gms./_____ ml.
= _____ gm./ml.

d = Vol./Wt. = _____ ml./ _____ gms.
= _____ ml./gm.

Large nail: d = Wt./Vol. = _____ gms./_____ ml.
= _____ gm./ml.

d = Vol./Wt. = _____ gms./_____ ml.
= _____ gm./ml.

## REPORT SHEET FOR EXERCISE 2 (continued)

3. Pipetting and Viscosity

| Trial | Water | Alcohol | Glycerol |
|---|---|---|---|
| 1 | _____ | _____ | _____ |
| 2 | _____ | _____ | _____ |
| 3 | _____ | _____ | _____ |
| 4 | _____ | _____ | _____ |
| 5 | _____ | _____ | _____ |
| Sum | | | |
| Mean | | | |
| Standard Error | ± | ± | ± |

Relative viscosity:

| | |
|---|---|
| Water | 1.000 |
| Alcohol | _____ |
| Glycerol | _____ |

Questions to be answered.

1. What is the difference between scales and a balance?

2. Is gravity the same all over the earth?

3. Why would it be that balances are used in scientific laboratories for weight determinations instead of scales?

4. How many grams in an ounce? _____
   How many grams in a pound? _____
   How many grams in a kilogram? _____
   How many pounds in a kilogram? _____

   How many centimeters in an inch? _____
   How many centimeters in a meter? _____

   A test tube may be 14mm. x 140mm. These measurements converted to inches would be:
   _____in. x _____in.

5. What particles of the atom account for most of its weight?

6. How much would 1 cc. of protons weigh?

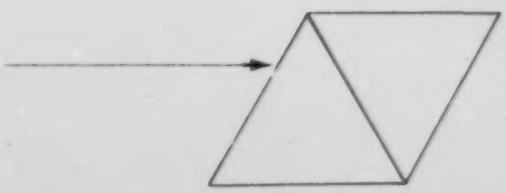
7. Distinguish between the mass of an object and its weight.

## EXERCISE 3 – DIFFRACTION OF LIGHT

### A. Diffraction by Prisms

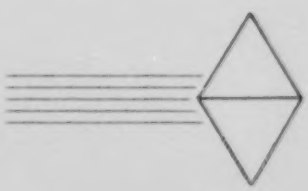
1. Arrange an intense light source, a tube or double slit in a box so that the column of light falls on a prism. Observe the spectrum obtained by placing a white card in the box as a screen. Answer questions on the answer sheet.

2. Now place a second prism next to the first so that the beam of light passes through both of them, viz:



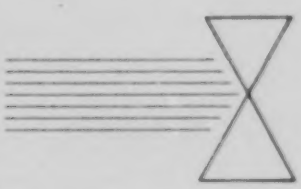
How would you explain the behavior of the beam of light?

3. Widen the size of the light beam and arrange the two prisms so that their bases come together in the midline of the beam. This would be the bar-shaped equivalent of a double convex lens.



Move the prisms closer to and farther from the screen. What is the effect?

4. Now arrange the prisms with their apices to each other in the beam of light.



## B. Diffraction Gratings

Diffraction Gratings are produced by cutting many fine, parallel grooves in a piece of glass or plastic. There are usually 10,000 to 15,000 such grooves per inch.

Because these can be molded in plastic such gratings can now be obtained relatively inexpensively, and are used in the student type hand spectroscopes provided in the laboratory. These consist of a paper tube with a slit in the cover on one end and the diffraction grating in the eyepiece on the other. When held to a light source, the spectrum is seen at about 45° or 60° from the line of sight to the slit.

Look at sunlight (not the sun) or a tungsten lamp. Is the spectrum seen uniformly bright and continuous?

Look at a mercury vapor fluourescent lamp. How does the spectrum differ from that seen from the white light of incandescent lamps?

Many uses are made in the laboratory of both the visible light spectrum and the invisible (to human eyes) spectrum in the infra-red and ultra-violet ranges. Instruments designed to measure the amount of light of specific colors absorbed by liquids is called a colorimeter if the wavelengths are intermittent (that is, determined by filters), or a spectrophotometer if a continuous spectrum of colors is available either from a prism or from a diffraction grating system.

Operation of the B & L Spectronic 20 spectrophotometer is described in Exercise 13.

## REPORT SHEET FOR EXERCISE 3

### A. Diffraction by Prisms

1. If red is number 1, what is the order of the other colors in the spectrum?

| | |
|---|---|
| 1 | red |
| _____ | blue |
| _____ | orange |
| _____ | violet |
| _____ | yellow |
| _____ | green |

Look up the colors produced by these wavelengths of light. Match.

| | Ångstroms | |
|---|---|---|
| _____ | 4200 | 1. red |
| _____ | 5600 | 2. blue |
| _____ | 6200 | 3. orange |
| _____ | 4600 | 4. violet |
| _____ | 5900 | 5. yellow |
| _____ | 5000 | 6. green |

2. How would you explain the behavior of the beam of light passing this arrangement of prisms?

3. What effect did this arrangement of prisms have?

## REPORT SHEET FOR EXERCISE 3

4. What effect did arranging the prisms with their apices together have on the beam of light?

B. Diffraction Gratings

1. Was the spectrum of sunlight and that from a tungsten lamp nearly the same?

2. How did the spectrum of a mercury vapor fluorescent lamp differ from that of a tungsten lamp?

3. What is a spectrophotometer?

## EXERCISE 4 – USE OF THE MICROSCOPE

The microscope is an important optical tool which makes visible details of structures, both biological and physical, which otherwise cannot be discerned with the naked eye. The cost of a microscope varies considerably, but in any case the investment is considerable. Proper handling assures good service from the instrument as well as reduces the probability of small disabling damages from occurring. This exercise, therefore, is aimed at introducing the student to the proper handling of the instrument and at the same time have him handle the microscope with confidence and assurance so that he can concentrate upon the material to be observed rather than worry about whether he is going to damage the instrument.

**Part A. Parts of the Classical Light Microscope**

Study the diagram on page 4-2 and identify the eyepiece, body tube, arm, objectives, condenser, and base.

Now read Part B, "Transporting the Microscope." By the time you have finished the teacher will ask you to identify the parts mentioned on a real microscope before you are asked to get the microscope assigned to you from the cabinet.

**Part B. Transporting the Microscope**

The microscope looks heavy and many of them are heavy. This feature is for steadiness when the instrument is in use and does not mean that the instrument is "rugged" in the usual sense.

1. *The microscope must not be jarred* as by dropping onto the floor or the table top. This will cause the lenses to be knocked out of alignment or even to break.

2. *Therefore, pick up the microscope by the arm (never by the body tube)* and support the base with your other hand.

3. *Carry the microscope upright.* Most microscopes have loose eyepieces which will fall out if the instrument is carried on a slant.

4. *Place the microscope upon the table gently.*

Now complete the identification of the parts of the microscope making use of the diagram on page 4-2 and answer the questions on the report sheets.

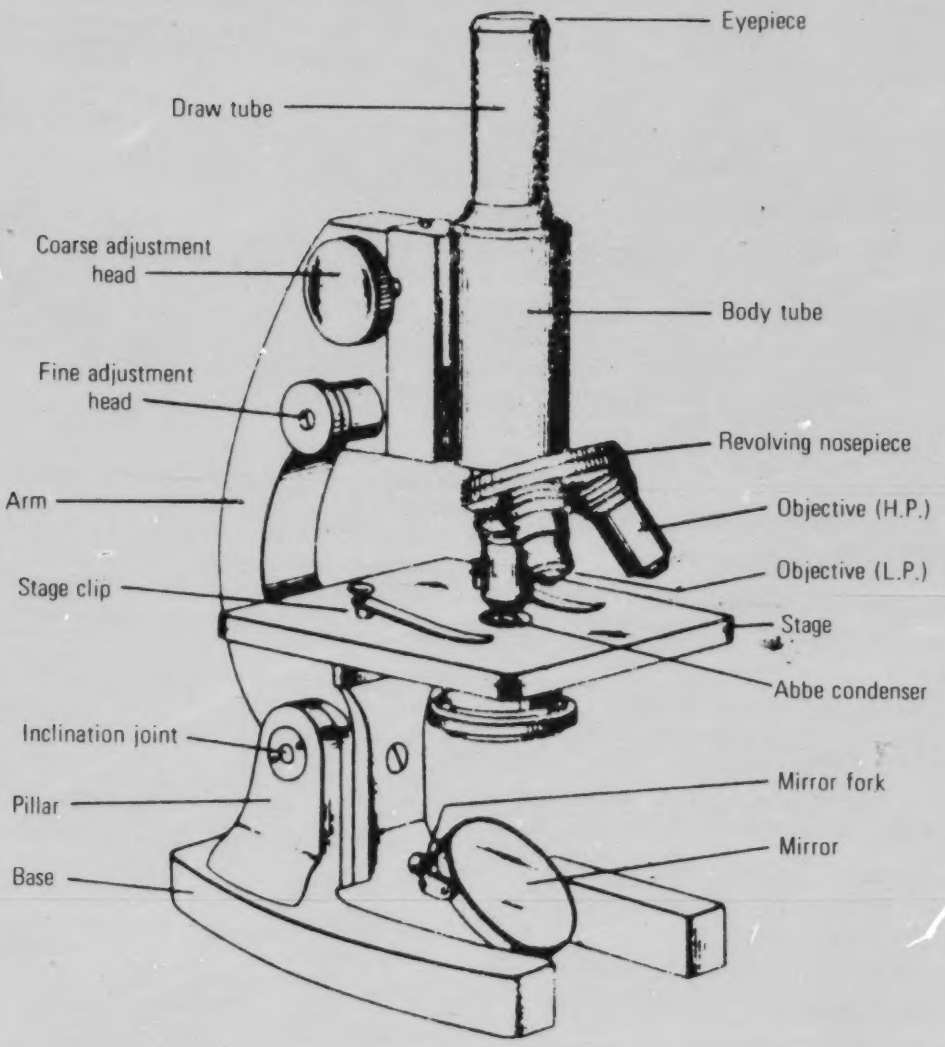
**Part C. Cleaning the Microscope**

1. Always use lens paper or optical tissue. Never use handkerchiefs, facial or bathroom tissue, paper towels, typing paper or emery cloth.

2. Wipe the glass surface, changing the place on the paper after each wipe. This is done so that if any grit is picked up it is not scrubbed back and forth over the lens. If there is an encrusted substance on the lens, wet a piece of lens paper with water and wash off the lens, then dry with lens paper. *Do not huff (blow) on the lens* to moisten it because small droplets of saliva come out with the breath and the protein it contains tends to leave smears on the glass.

The surfaces to be cleaned are the:
- upper surface of the eyepiece
- objective lenses
- upper surface of the condenser
- mirror

## Parts of the Compound Microscope




Courtesy of the Bausch and Lomb Optical Company

**Part E. Making an Observation**

1. Put the low power objective (marked 1.6 cm.) in place. A spring clip seats the nosepiece exactly so that the lenses are in line with the eyepiece. Use the course adjustment head to bring the bottom of the objective about 1.6 cm. (½ inch) above the stage.

2. If your microscope has a condenser, adjust the plain side of the mirror so that light is directed through the condenser. If your instrument does not have a condenser, use the concave side of the mirror in order to focus light at the level of the stage.

3. Raise the condenser to stage level. Look through the eyepiece and adjust the iris diaphragm on the condenser or under the stage so that the lighted field is about the same size as the visible field.

4. Place a slide containing a 2 mm. printed scale on the stage and secure it with the spring clips or mechanical stage apparatus. Place the slide so that the part to be observed is in the light. Adjust the focus with the course adjustment head so that the object is distinctly seen.

How does the image move in the field compared with the way that the slide is actually moved on the stage? (Answer on the report sheet.)

5. Measure the diameter of the field to the nearest millimeter (mm.). Convert the mm. to microns by multiplying by 1000 (1000 microns = 1 mm.).

6. Compute the magnification of the low power system by multiplying the rated magnification of the ocular (usually it is 10X, but check yours to be sure) by the rated magnification of the low power objective (usually 10X, but check again to be sure).

7. Now rotate the nosepiece to bring the high-dry objective (4 mm.) into place. The high-dry objective is usually rated about 45X and is marked for working about 4 mm. above the slide. Focus *upward* with the fine adjustment head. If the scale does not come into focus your microscope is not *parfocal*, if it does, it is. (There is nothing you can do about this now.) To find the object, watch from the side and lower the objective to a position near the slide (2-3 mm. above it), but not touching it. Now look through the eyepiece and focus upward with the fine adjustment head.

The course adjustment head should not be used for focusing high-dry and oil immersion objectives. Adjust the iris diaphragm so that the light just covers the field of view. High-dry and oil immersion require increasing amounts of light. Why?

It is good technique to keep both eyes open. With a little practice you can mentally repress the vision in the eye not being used for microscopy, but at the same time you avoid the strain of keeping eyelid muscles contracted for long periods of time resulting in muscle fatigue and possibly a little pain.

8. Measure the width of the field, estimating the last fraction of a mm. Convert your measurements to microns and record them on the report sheet.

9. Repeat Steps 7 and 8 for oil immersion objective, if there is one on your microscope. Perform these steps:

   a. Add a drop of immersion oil to the part of the slide to be observed.

   b. Lower the objective until it touches the drop of oil. You will see a "flash of light" as the drop changes shape.

   c. Focus upward with the fine adjustment head.

10. Complete the computations on the answer sheet.

**Part F. Computing the Resolution**

Magnification is of importance but of equal importance is the sharpness of the image. This is the factor called *resolution*. It is actually defined as the ability to separate visually two closely placed points. This is a function of lens structure called its *numerical aperture (N.A.)* and the wavelength (color) of the light being used. Below is a nomogram which relates these factors. Compute the resolution for each objective lens system on your microscope.

**Part G. Depth of Focus and Plane of Focus**

1. Obtain a slide of a tissue containing a lot of similar cells, such as a slide of mammalian liver or kidney, or a slide containing mostly a simple epithelium.

2. Put the low power objective in place. (Always begin observations with low power before going to high magnifications.) Bring the slide into focus and adjust the light. You will note some numbers on the fine adjustment head. See how many numerical units are included between being in focus at the bottom of the tissue and at the top of the tissue (bottom and top with respect to the objective lens).

3. Now go to high-dry. Is the whole depth of the tissue cells, which are about 10 microns thick (more or less) in focus as at one time? Is all of the nucleus of a cell in focus at once? When the bottom of the nucleus is in focus is the top of it in focus? When the bottom of the cell is in focus is the top of it in focus? How many numerical units on the fine adjustment head are there between having the bottom of the cell in focus and the top of it?

The plane of focus is usually flat. The depth of focus is approximately the same at the various magnifications and refers to the depth of the plane in focus.

**Part H. Using the Zoom Microscope**

If you are to use one of the more simplified microscopes, you will find the instructions for a classical light microscope inadequate. On page 4-7 you will find a diagram of the B & L Academic 225 Student Zoom microscope. The basic difference between this and microscope illustrated on page 4-2 is that there is no nosepiece and therefore only one objective which changes in magnification as it moves up or down. This microscope cannot be focused manually, for it has a harmonic drive fine focusing mechanism which cannot be moved out of adjustment. It thus provides a continuous gradation of magnification by the objective between 10X and 50X. The eyepiece is a 10X widefield lens system. The Numerical Aperture is 0.55.

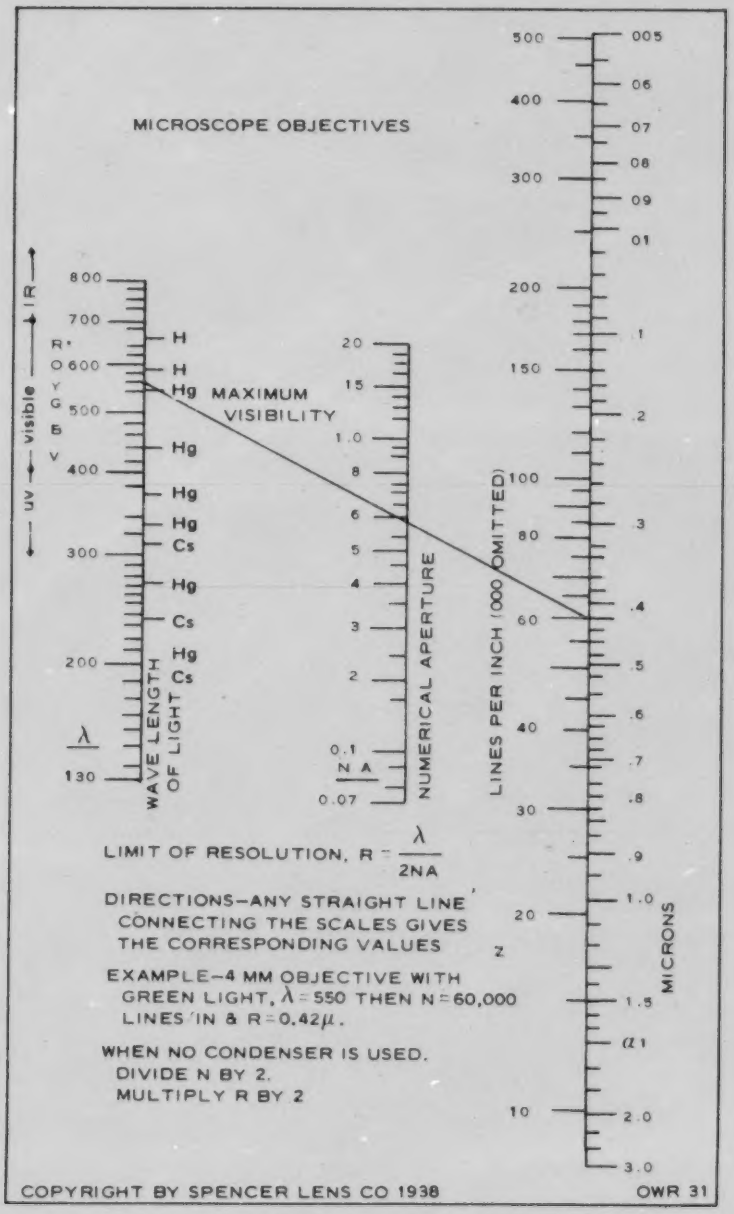
Although it is built of sturdy, lightweight aluminum construction, it nevertheless must be handled carefully or the lens system will be jarred out of focus. There is a built-in pointer.

Transport the microscope as described in Part B.
Clean the microscope as described in Part C. Surfaces to be cleaned are the:
- upper surface of the eyepiece
- objective lens
- upper surface of the light source.

Making an Observation

1. Raise the objective to its highest position.
   Place a mm. rule or a stage micrometer on the stage over the condenser port and secure with the clips.
2. Measure the width of the field at the lowest power and again at the highest power.
3. Divide the high power into the low power measurement and multiply by 100. How does this figure compare with the rated magnifications on the instrument?

# Nomogram for Determining the Resolution of a Lens System




*Nomogram for the resolving power of microscope objectives.*
*(Tr. Am. Micro. Soc., 1938, 57:316-318)*

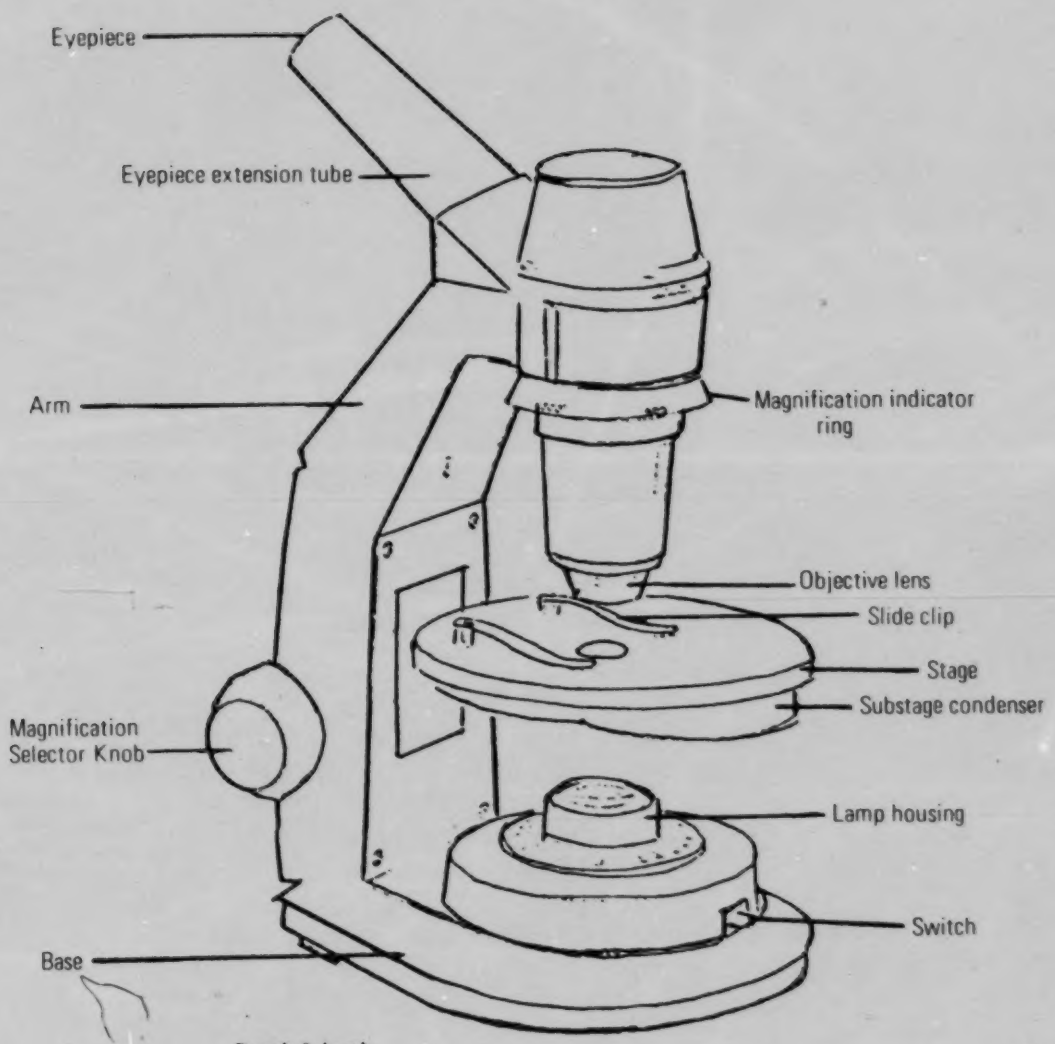
*R = RED, O = ORANGE, Y = YELLOW, G = GREEN, B = BLUE, V = VIOLET

**Part I. Using the Stereoscopic (Dissecting) Microscope**

This is a low power instrument that has a 10X eyepiece (as a rule) and objectives ranging from about .7X to 3X magnification. Most work with this instrument is by reflected light, that is the object to be studied is lighted from above. The two eyepieces should be adjusted for the distance between your eyes. When you look through the eyepiece with each eye singly the image should be in focus (after you have focused with the focusing knob). If the image is in focus for one eye but not the other, turn the eyepiece adjustment band until it is. When the distance between eyes is properly spaced, the field should appear larger than with either eye singly.

Is the image reversed?

Look at a slide of tissue, such as one of liver. Can you see cellular details? Examine a penny.




Bausch & Lomb
ACADEMIC 255 Student
Zoom Microscope MODEL 011

REPORT SHEETS FOR EXERCISE 4 Name _____

Section _____ Date _____

**Part A. Observations on the Parts of the Microscope**

1. Put the following in the correct order in which light passes on its way from the lamp to the eye by numbering 2, 3, 4, etc.

| | |
|---|---|
| 1 | lamp |
| _____ | nosepiece |
| _____ | eyepiece lenses |
| _____ | Abbe' condenser lens |
| _____ | condenser iris |
| _____ | objective lenses |
| _____ | body tube and draw tube |
| _____ | mirror |

2. Where is the iris diaphragm control located? _____

_____

3. Where is the condenser-raising head located? _____

4. Where is the course adjustment head found on your microscope? _____

_____

**Part E. Making An Observation**

5. How does the image move in the field compared with the way that the slide is actually moved on the stage?

_____

7. Why must one focus *upward* on higher powers of magnification? _____

_____

Why does high-dry and oil immersion magnifications require more light than low power?

_____

_____

**Part G. Resolution**

3. Is the bottom of the nucleus in focus when the top of it is? _____

Is the top of the cell in focus when the bottom is? _____

To what use can the numbers on the fine adjustment head be put? _____

_____

## REPORT SHEETS FOR EXERCISE 4

Name _____

Section _____ Date _____

What is the number of your microscope? _____

### Rated Magnification

| OBJECTIVE LENS | RATED MAGNIFICATION OF OBJECTIVE | RATED MAGNIFICATION OF EYEPIECE LENSES | TOTAL RATED MAGNIFICATION |
|---|---|---|---|
| Low Power | | | |
| High-dry | | | |
| Oil Imm. | | | |

### Calibration (Real Magnification)

| OBJECTIVE LENS | RATED WORKING DISTANCE | DIAMETER OF FIELD IN mm. | DIAMETER OF FIELD IN microns | MAGNIFICATION IF LOW POWER IS 100X* |
|---|---|---|---|---|
| Low Power | | | | 100X |
| High-dry | | | | |
| Oil Imm. | | | | |

*Divide the microns at High-dry and Oil Immersion into the microns across the Low Power field and multiply by 100. For example: $1500\mu / 1500\mu \times 100 = 100X$. Or,

$1500\mu / 375\mu \times 100 = 4 \times 100 = 400X$.

### Resolution (Determined from nomogram)

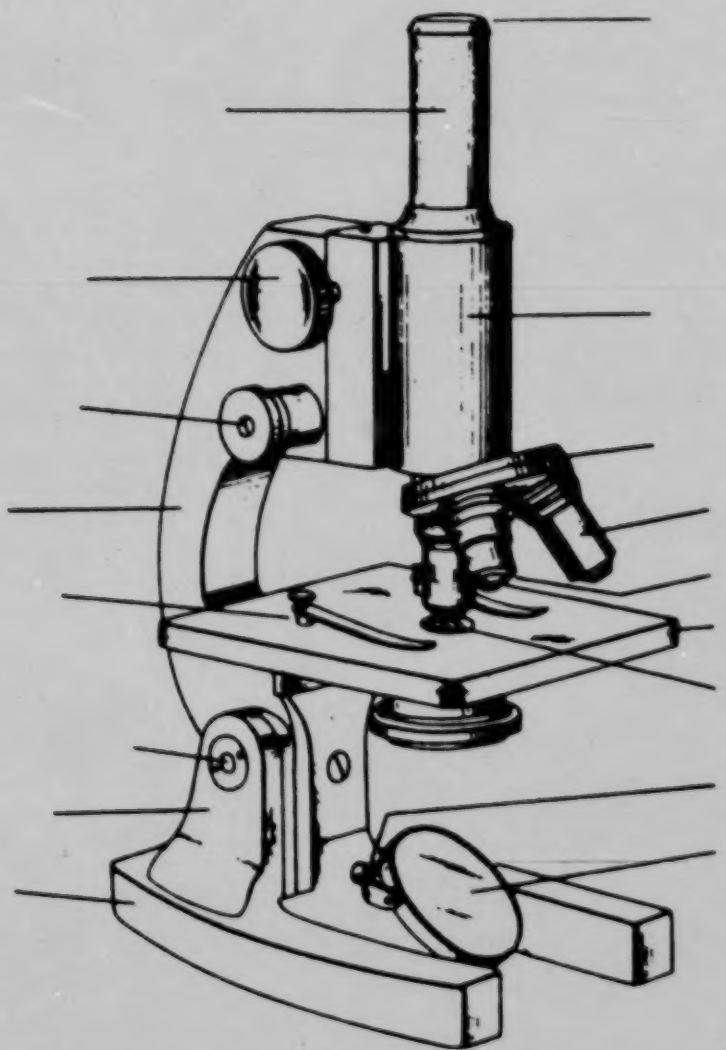
| OBJECTIVE LENS | RATED N.A. | COLOR OF LIGHT | RESOLUTION IN MICRONS |
|---|---|---|---|
| Low Power | | | |
| High-dry | | | |
| Oil Imm. | | | |

Name _____

Section _____ Date _____

## SELF-TEST ON THE MICROSCOPE

See how well you can name the indicated parts of the microscope without referring to the chart on page 4-2. Afterwards check your answers with the diagram and review those parts you missed. Correct any errors.



Courtesy of the Bausch and Lomb Company

## REPORT SHEET FOR EXERCISE 4

### Part H. Using the Zoom Microscope

What is the number of your microscope? _____

| RATED MAGNIFICATION OF EYEPIECE LENS | INDICATED MAGNIFICATION | IMPLIED MAGNIFICATION OF OBJECTIVE LENSES | |
|---|---|---|---|
| 10X | _____ | _____ | (Lowest power) |
| 10X | _____ | _____ | (Highest power) |

Calibration of the Field (Real Magnification)

| | DIAMETER OF FIELD IN mm. | DIAMETER OF FIELD IN microns | MAGNIFICATION IF LOWEST POWER IS 100X? |
|---|---|---|---|
| Lowest power | _____ | _____ | 100X |
| Highest power | _____ | _____ | _____ |

Resolution

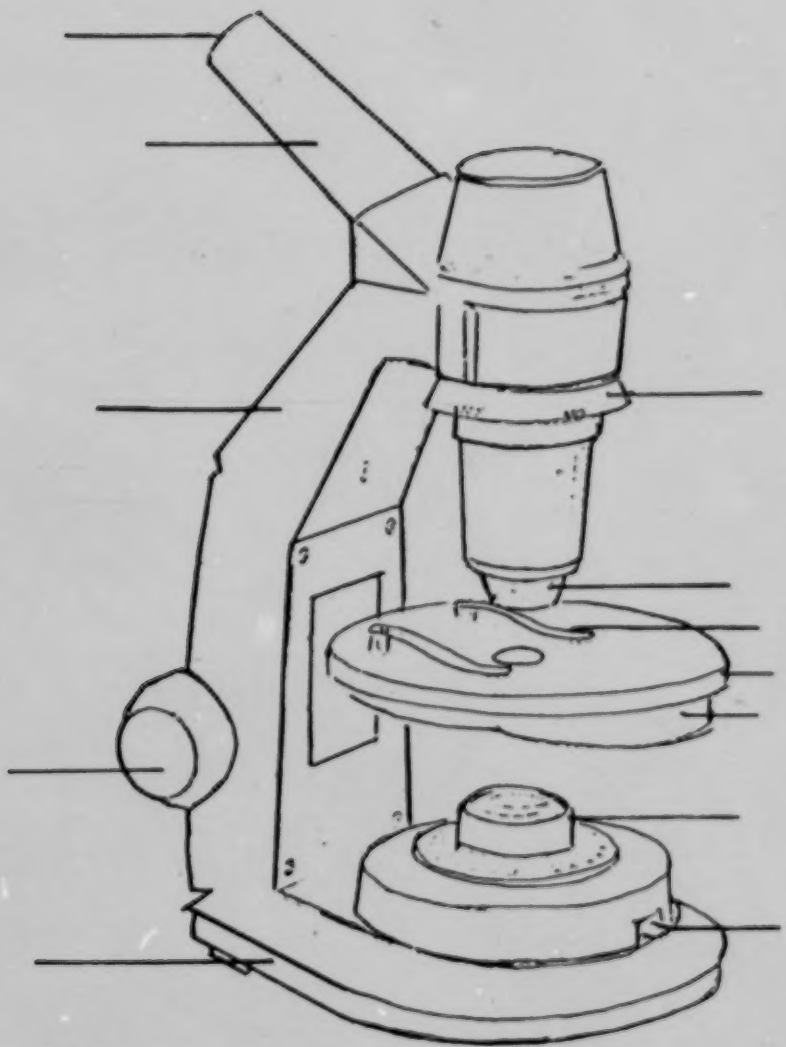
| | RATED N.A. | COLOR OF LIGHT | RESOLUTION IN MICRONS |
|---|---|---|---|
| Lowest power | _____ | _____ | _____ |
| Highest power | _____ | _____ | _____ |

### Part I. Using the Steroscopic (Dissecting) Microscope

1. Is the image reversed?
2. How did cells in a slide of tissue look?

   How did a penny look?

## Self Test for Zoom Microscope



Bausch & Lomb
ACADEMIC 255 Student
Zoom Microscope MODEL 011

## EXERCISE 5 – STERILIZING AND STERILE TECHNIQUE

This is a combination of exercises and experiments to introduce the student to some of the usual techniques used in producing and maintaining asceptic (sterile) conditions in certain containers and objects such as food tins and jars, surgical dressings, rocket parts, drugs, and baby bottles. Corollary to these techniques is to know what the difference is between being chemically clean and biologically sterile. In this exercise living microbes will be killed with a chemical poison (mercuric chloride), with moist heat in a pressure cooker or autoclave, and by incineration in a flame.

### Part A. Making Sterile Transfers With A Wire Loop

Material and Equipment (per student)

- 1 Bunsen burner
- 1 Nichrome wire transfer loop with handle
- 1 Cellulose Sponge or paper towelling
- 1 Test tube rack
- 1 Clean test tube
- 3 tubes containing 5 ml. of sterile nutrient broth

For each table

- 1 culture of *Escherichia coli* or *Aerobacter aerogenes*
- 1 bottle (gallon) of .1% mercuric chloride (marked POISON)

Procedures

Preparation

1. Wipe off the work area with 0.1% mercuric chloride solution using a sponge or paper towelling. Leave the surface damp, not wet, so that it will dry quickly in air. Wash your hands.

2. Place before you on the now self-sterilizing work surface, a test tube rack with a clean tube half full of tap water and three tubes containing 5 ml. each of sterile nutrient broth, a nichrome wire with a loop 2-3 mm. in diameter and mounted in a handle, and a bunsen burner with a blue flame about 6 inches high.

Flaming Tubes

3. If you are right-handed, take a tube of sterile broth in your left hand. For maximum maneuverability press the tube against the tips of four fingers with the thumb. Hold the tube on a slant.
Remove the tube closure with the back of the 4th and 5th fingers of the right hand.
Keeping the tube slanted, flame one side of the tube and then the other for about 1 second (count one-thousand-one).

4. Pick up the nichrome wire with the right hand. *For this time only* do not flame the wire, but put it into the broth and jiggle it about a bit.
(Why was the tube carefully flamed if an unflamed wire was to be used?)

5. Again flame the mouth of the tube.
Replace the tube closure.
Label the tube with your name and A-1.
Replace the tube in the test tube rack.

Flaming the Transfer Loop

6. Dip the wire loop into the tube of tap water then plunge it into the flame.
   Is there any splattering (or spluttering)? What would be the consequences if the liquid were from a culture of dangerous organisms instead of water?

7. Dip the wire into the water again.
   Hold it at about a 30° angle from the vertical *above* the flame to *dry* it.
   Now lower the wire into the flame at about the same angle and bring it to red heat. The whole wire must be heated.
   Allow the wire about 15 seconds to cool.
   Touch it to the water in the tube. If it sizzles, reheat the wire and wait a little longer time. Touch it to the water. Continue this procedure until you find out how long it takes the wire to cool down enough to not cause the sizzling sound on contact with the water.

Is the Loop Sterile?

8. Take a tube of sterile broth in the left hand.
   Flame the nichrome wire loop and let it cool.
   While the loop cools, remove the closure from the tube of broth and flame it as in Step 3.
   Plunge the wire into the broth, jiggle and remove it, but do not lay the wire down.
   Flame the mouth of the tube and replace the closure.
   Replace the tube in the rack.
   Dry and flame the wire.
   You may now lay the loop down on the work surface.
   Now label the tube with your name and A-2. Label the unused tube A-3.

9. Observe Tubes A-1 and A-2 in 24 hours at room temperature and record your results on the Report Sheet. Also observe Tube A-3.

Asceptic Transfer of Organisms With the Wire Loop

Each species and strain of bacteria can frequently be distinguished by the kinds of colonies it produces and by the kind of biochemical products that it makes. E. coli and A. aerogenes belong to the sub-group of bacteria known as enteric bacteria. Members of this group can frequently be distinguished by the reactions of their products to tests for indole, acid (as measured with methyl red), acetyl methyl carbinol, and the ability to use citrate as its sole source of carbon. These tests are therefore referred to as the IMViC tests. If your transfers are done asceptically and without contamination of other organisms, then your tests made on these transfers should be consistent with those determined for the strain.

In this section innoculate two tubes of glucose peptone water and one of Koser's citrate.

10. Hold the tube of pure culture and a tube of sterile medium both in the left hand.

11. Flame the wire loop. While it is cooling remove the tube closures from both tubes (held at an angle (with regard to the vertical), using the 4th and 5th and the 2nd and 3rd fingers.
    When the wire has cooled (so as to not kill the bacteria in the culture), collect a loopful of culture and transfer it to the tube of sterile broth.
    Flame the tubes again and replace the right closure to the right tube.
    Replace the tubes in the tube rack.
    Dry and flame the wire loop.
    Innoculate the other two tubes (one of glucose peptone water and one of Koser's citrate) using the same technique.

Label the tubes with your name, the name of the bacterium, and the medium in the tube.

13. Observe the cultures in 24 to 48 hours and perform the tests below:

Citrate. Did the organism grow in this medium?

Indole Production. Add 1-2 ml. of ether to a culture in glucose-peptone water. Mix by rotating between the palms of the hand. Let the ether separate out. Then add down the side of the tube about half as much Kovac's reagent* as there is culture. A red color in the ether layer indicates the presence of indole.

*(Kovac's reagent contains amyl or butyl alcohol, 75 ml.; concentrated HCl, 25 ml.; and para-dimethyl-amino-benzaldehyde, 5 gm.)

Methyl Red Test. With a sterile pipette (see sections which follow) transfer 1 ml. of a culture in glucose-peptone water to a clean test tube. Add a few drops of methyl red. This indicator has a pKa of 5.0, being red below and yellow above that pH.

Voges-Proskauer Reaction for Acetyl Methyl Carbinol. From the remainder of the culture in glucose-peptone water used for the methyl red test remove 1 ml. to a clean test tube. Add 0.6 ml. of 5% alpha napthol in absolute alcohol and 0.2 ml. of 40% KOH. The development of a crimson to ruby red color in 2 to 4 hours is a positive test for this product. However, the color fades so the test should be evaluated not longer than 4 hours after mixing the reagents.

Record your results on the report sheet.

**Part B. Making and Autoclaving Media**

Litmus Milk

2 sterilized nursing units containing sterile litmus milk
1 empty nursing units
Test tube brush and detergent
Bunsen burner and wire loop
Triple beam balances
100 ml. graduated cylinder
Culture of *E. coli*

Procedure

1. Wash the empty nursing bottles thoroughly with detergent and rinse completely under running tap water. Let drain on a clean towel.

2. Weigh out 10 grams of dry litmus milk and add to each of the clean bottles. Then add 100 ml. of tap water. Replace the nipple and closure ring and shake to mix. Label the bottles with self-adhesive labels numbered 1 and 2 and containing your name(s) and laboratory day. Place bottle No. 1 on the tray on the teacher's table to be autoclaved. Place bottle No. 2 on the reagent shelf over your workspace.

3. Label the two bottles of sterilized litmus milk 3 and 4 plus the other information on the other labels. Open bottle No. 3 and transfer a loopful of a pure culture of E. coli using the asceptic technique used in Part A of this exercise. Reclose the bottle and place both bottles on the reagent shelf.

4. Come back in one and two days to record color changes in any part of the bottle. Bottle No. 1 will be sterilized and made available to you by tomorrow.

Autoclaving is done either in a pressure cooker or in an autoclave at 15 pounds per square inch (psi) steam pressure (126°C.) for 15 minutes for ordinary test tubes and flasks under 600 ml. Larger volumes require a longer autoclaving time. Steam pressure may not be released suddenly

from liquids. They must be allowed to cool below 100°C. before pressure is released or the boiling of the liquid will usually blow the closure off of the tube or flask, leaving it exposed for contamination.

**Part C. Making a Pipette Transfer and Spreading a Petri Plate**

1 tube of E. Coli
1 empty sterile test tube
2 Bromthymol Blue-agar petri plates
1 sterile 10-ml. sterile pipette

Procedure

1. Open the mouth end of a bag of sterile 10-ml., cotton-plugged pipettes. Remove one without letting the lower part make contact with the opening of the bag and it should not contact any other non-sterile surface.

2. Take the tube of E. coli culture in the left hand and remove the cap with the 4th and 5th fingers of the right hand. Insert the pipette and withdraw 6 ml. Recap.

3. Lift the cover of the Bromthymol Blue petri plates not more than to 45° on one side and pipette in .5 ml. of buffer, being careful not to let the pipette touch the edge of the petri dish. Lower the covers. Label them C1 and C2. Discard pipette.**

4. Try spreading the liquid in plate C1 by tilting the plate so that the fluid moves over the surface. However, be careful to NOT let the liquid come in contact with the upper edge of the plate side or it will offer an avenue for the inflow of organisms at the edge of the cover.

5. Make a spreader. Take a Pasteur pipette and seal the end in a flame, and then make about a 45° bend about 1.5 inches from the end. Let cool.

6. Dip the spreader into 95% ethanol and light in a flame. Hold away from the flame and let the alcohol burn out. Let cool for a few seconds. Raise the edge of petri dish C2 about 45° and spread the puddle of buffer evenly over the surface with the spreader.

**Part D. Making a Serial Dilution and Plating**

Culture of E. coli, O.D. .25 at 620 millimicrons
6 1-ml. sterile pipettes
6 250-ml. flasks containing 99 ml. water, plugged, foil-covered, and sterilized
8 Petri dishes with plate count agar

Procedure

1. Label the flasks with wax pencil $10^{-2}$, $10^{-4}$, $10^{-6}$, $10^{-8}$, $10^{-10}$ and $10^{-12}$

2. On the label side of the petri dish cover write your name, laboratory day, and $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$, $10^{-10}$, $10^{-12}$

3. Take the $10^{-2}$ flask. Lift the aluminum foil enough to remove it as a cap from the flask. Keep it top side up and place it on the table top. Remove the plug and discard on the table top. Open a sterile 1-ml. pipette casing at the mouth end and remove the pipette. Take the culture of E. coli in the left hand and remove exactly 1.0 ml. of culture with the pipette. Reclose the tube. Transfer the E. coli to the flask, rinsing the pipette out 3 times with the contents. Flame the underside of the aluminum foil cover and replace it on the flask. It should not be flamed too long or it will melt and

** Discard used pipettes into the pipette jars located in the middle of the work table. In doing so insert the pipette into the detergent solution with the tip DOWN.

leave a hole in the foil. Take the flask in the right hand and shake it through an arc of 45° about 20 times to thoroughly mix the contents. This is a very important maneuver since the outcome depends of having a very homogenous suspension of bacteria. Discard the used pipette.

4. Take a new sterile 1-ml. pipette, the $10^{-4}$ flask and the $10^{-3}$ plate.

5. Transfer 0.1 ml. of the $10^{-2}$ suspension to the $10^{-3}$ plate, being careful not to touch it. (Let your partner flame a glass spreader and spread it.). Now, using the same pipette, transfer 1.0 ml. of the $10^{-2}$ suspension to the $10^{-4}$ flask. Discard the pipette in the pipette jar. Flame the cover to the $10^{-4}$ flask. Replace it. Shake 20 times through a 45° arc.

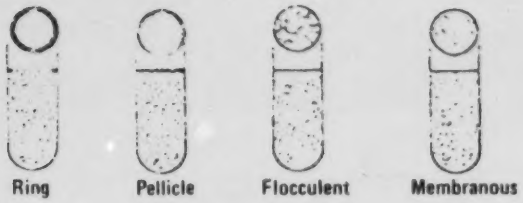
6. Take a new sterile 1-ml. pipette, the $10^{-6}$ flask, and the $10^{-4}$ and the $10^{-5}$ plates. Transfer 1.0 ml. of the $10^{-4}$ suspension to the $10^{-4}$ plate and 0.1 ml. to the $10^{-5}$ plate. (Have your partner flame the spreader and spread one plate, then flame it again before spreading the next. Be sure to let the spreader cool enough so that it does not melt the agar.) Transfer 1.0 ml. of $10^{-4}$ to the $10^{-6}$ flask. Flame the aluminum foil cover, replace it and shake the flask 20 times through a 45° arc.

7. Continue the process. Remember that the transfer of 1.0 ml. of a suspension goes to the plate with the same dilution factor as the suspension. Transfer of .1 ml. of suspension goes to a plate with the next highest negative exponent number.

8. Let the plates rest upright on the table top until all of the fluid has been absorbed by the agar, then invert the plate to conserve moisture. Leave inverted at the back of the workspace for 18 to 24 hours at room temperature, then count the colonies according to the rules below.

9. Sterilize the dilution flasks by pouring in about 50 ml. (.5 volume) of 0.1% mercuric chloride. Let stand at least 10 minutes, then pour down the sink hole (not into the lead troughs or splashed into the sink). Rinse out the flasks.

## Characteristics of Broth Cultures




1. Surface
   - none
   - ring
   - pellicle, bacterial growth forming either a continuous or an interrupted sheet over the culture fluid
   - flocculent, containing small adherent masses of bacteria of various shapes floating in the culture fluid
   - membranous

## Characteristics of Agar Plate Cultures

Form

- punctiform, very small, but visible to naked eye; less than 1 mm in diameter

- circular

- filamentous, growth composed of long, irregularly placed or interwoven threads

- irregular

- rhizoid, growth of an irregular branched or rootlike character

- spindled, larger at the middle than at the ends

Elevation

- flat

- raised, growth thick, with abrupt or terraced edges

- convex

- pulvinate, cushion-shaped

- umbonate

Margin or edge

- entire

- undulate

- lobate, having lobes, or rounded projections

- erose, irregularly notched

- filamentous, growth composed of long, irregularly placed or interwoven threads

- curled, composed of parallel chains in wavy strands

## REPORT SHEET FOR EXERCISE 5

### Part A. Making Sterile Transfers With A Wire Loop

| TUBE AND MEDIUM | ORGANISM INNOCULATED | GROWTH | METHYL RED | VOGES-PROSKAUER | INDOLE |
|---|---|---|---|---|---|
| A-1 Nutrient Broth | Non-sterile Loop | | XX | XX | XX |
| A-2 Nubrient Broth | Incinerated Loop | | XX | XX | XX |
| Koser's Citrate | | | XX | XX | XX |
| Glucose-Peptone | | | XX | XX | |
| Glucose-Peptone | | | XX | | XX |
| Glucose-Peptone | | | | XX | XX |
| Teacher's Control Tests for This Organism | | | | | |

Write a paragraph which answers these questions:
What is a correct interpretation of the experiment involving Tubes A-1 and A-2?
Why was Tube A-1 flamed if a non-sterile loop was going to be inserted?
Why have a tube like Tube A-3 involved in the experiment?

## REPORT SHEET FOR EXERCISE 5

**Part B. Making and Autoclaving Media**

Section 1–Litmus Milk

| BABY BOTTLE NUMBER | SOURCE AND TREATMENT | COLOR AT START | COLORS AFTER | |
|---|---|---|---|---|
| | | | 24 HOURS | 48 HOURS |
| 1 | Your own mix Sterilized | | Top<br>Bottom | |
| 2 | Your own mix Not sterilized | | Top<br>Bottom | |
| 3 | Provided sterile E. coli added | | Top<br>Bottom | |
| 4 | Provided sterile No treatment | | Top<br>Bottom | |

Indicate with the letter R if rennet (curd and whey) were formed after 24 or 48 hours.

1. What is the source of litmus? What is its pKa as an indicator dye?

2. Did you observe any decoloration? _____ Why would this occur?
(If decoloration did not occur in your own bottles look at someone else's where it did and answer the question.)

3. What is the relative volume and consistency of the curd? What change in the bottle brings about what change in the milk protein to produce this effect?

4. Did you consider the washed bottles to be clean enough for you to drink from?
What were the possible sources of bacteria in bottle 2?

5. After being autoclaved at 15 psi for 15 minutes is litmus milk capable of supporting microbial growth? _____ What is the basis for your answer?

6. What is meant by the control group or sample in an experiment?

## REPORT SHEET FOR EXERCISE 5

### Part C. Making a Pipette Transfer and Spreading a Petri Plate

Results:

1. Did the 5 ml. of buffered broth transferred to the sterile tube remain sterile?

2. Did the loop of E. coli grow in the innoculated sample of broth?
   Was one of these forms of surface growth seen? Ring Pellicle Flocculent Membrane

3. What would be the disadvantages of an uneven spread if you were going to have to make bacterial colony counts?

### Part D. Making a Serial Dilution and Plating

| DILUTION OF SUSPENSION | ML. USED | DILUTION ON PLATE | NO. OF COLONIES ON THE PLATE | BACTERIA/ML. |
|---|---|---|---|---|
| $10^{-2}$ | .1 | $10^{-3}$ | | |
| $10^{-4}$ | 1.0 | $10^{-4}$ | | |
| $10^{-4}$ | .1 | $10^{-5}$ | | |
| $10^{-6}$ | 1.0 | $10^{-6}$ | | |
| $10^{-6}$ | .1 | $10^{-7}$ | | |
| $10^{-8}$ | 1.0 | $10^{-8}$ | | |
| $10^{-10}$ | 1.0 | $10^{-10}$ | | |
| $10^{-12}$ | 1.0 | $10^{-12}$ | | |

What were the characteristics of the colonies?

Form: Punctiform circular filamentous irregular rhizoid spindled

Elevation: flat convex pulvinate, cushion-shaped umbonate

Margin or edge: entire undulate lobate erose filamentous curled

Why do you suppose that colonies are smaller on crowded plates than they are on uncrowded plates?

Will every viable bacterium on the plate give rise to a discrete colony?

# EXERCISE 6 – BACTERIAL MUTATIONS[1]

## Introduction

Since evolution is a dynamic process involved intimately with the mechanism of natural selection a good demonstration of this mechanism can be observed directly. By selecting an outstanding trait or character of an organism one can determine by direct means the presence or absence of the character after some external factor is applied to the organism.

Ultraviolet light has been used as a substitute for certain natural selective forces to produce genetic mutations in bacterial cultures. Therefore, ultraviolet light should produce reproducible alterations in a bacterial culture that can be readily followed through successive generations.

Materials and Equipment

Macalaster Ultraviolet Germical Lamp – 2537 Angstroms

Media:

Milk, nutrient broth or trypticase glucose broth, trypticase glucose agar slants and bottles.

Sterile Equipment:

Pipettes–10.0 ml, 5.0 ml, and 1.0 ml graduated in 1/100 ml.
Disposable sterile Petri plates
Bent glass rod spreader
Test tubes–18 mm or 16 mm.
Water blanks–distilled water, 9.9 ml/tube and 9.0 ml/tube

Incubation chamber or room at 30°C and 37°C

*Culture*

This organism is in the coliform group of bacteria and is a common soil saprophyte. Some main characteristics are aerobic growth, peritrichous flagella, gram negative, rod-shaped, and carries out a butylene glycol type end-product metabolism. It also has one outstanding characteristic. It produces a red pigment when grown at 28-30 degrees C. NAME: *Serratia marcescens.*

NOTE: The plates of agar medium should be prepared and stored at 37°C for at least 48 hours before use in order that the medium will be sufficiently dry to prevent coalescence of colonies.

## Procedure

*Preparation of Culture*

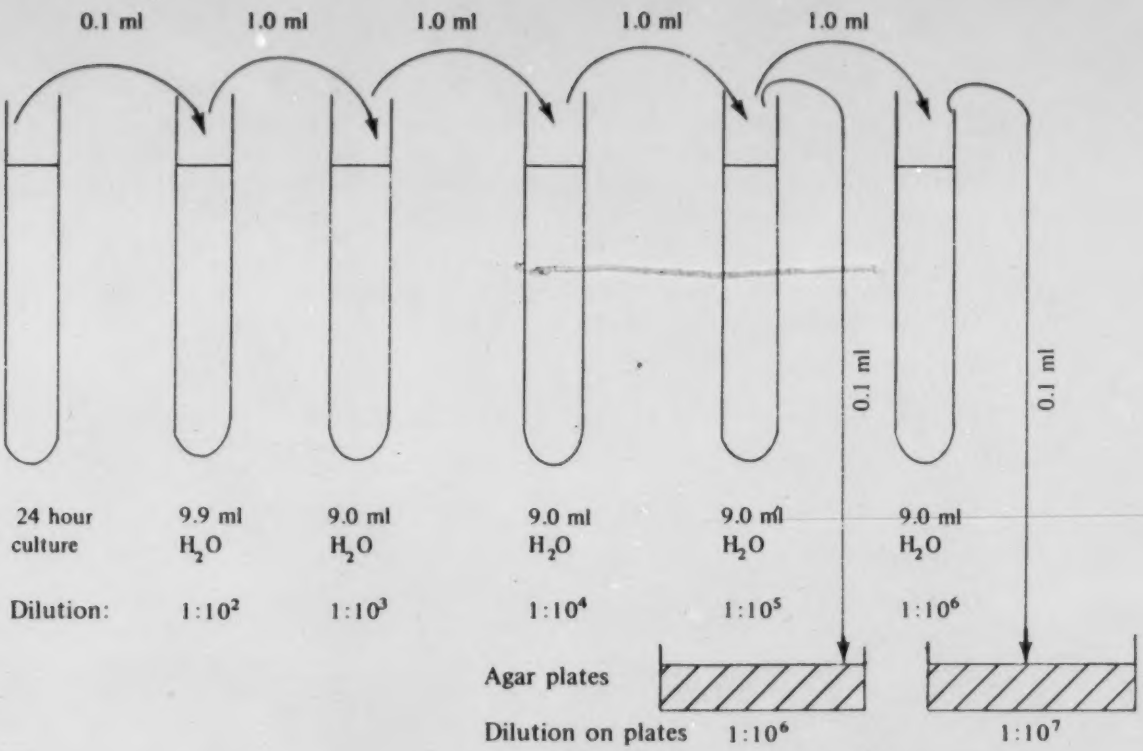
Inoculate 3 tubes containing approximately 10 ml of liquid growth medium–nutrient broth or trypticase glucose broth–from a 24-hour agar slant culture of *Serratia marcescens.*

Incubate at 30°C for 24 hours.

Transfer 10 ml of the 24-hour culture to a sterile Petri dish. Withdraw a 0.1 ml aliquot and transfer it to a sterile 9.9 ml distilled water blank. This is an initial dilution of 1:100. Make subsequent 1.0 ml dilutions in transfers to 9.0 ml sterile distilled water blanks until a 0.1 ml aliquot will give a $1:10^6$ and $1:10^7$ dilution when plated on the surface of agar.

[1] Courtesy Dr. Paris M. Allen, ISE Staff

Dilution example:




Spread the suspension evenly over the agar surface by means of a bent glass rod. This is the zero time irradiation specimen.

Turn the UV lamp on approximately 10 min. before use. Irradiate the remaining bacterial suspension in the Petri plate for 3 minutes, withdrawing 0.1 ml. aliquots at 30-second intervals. Plate the following dilutions for each time interval.

| Time of Irradiation | Dilution Plated | | |
|---|---|---|---|
| 30 sec. | $1:10^4$ | $1:10^5$ | $1:10^6$ |
| 60 sec. | $1:10^3$ | $1:10^4$ | $1:10^5$ |
| 90 sec. | $1:10^2$ | $1:10^3$ | $1:10^4$ |
| 120 sec. | $1:10^1$ | $1:10^{2*}$ | $1:10^3$ |
| 150 sec. | $1:10^1$ | $1:10^{2*}$ | |
| 180 sec. | $1:10^1$ | $1:10^{2*}$ | |

*Take a 1 ml. aliquot of the irradiated culture and dilute in 9.0 ml. of water to obtain a $1:10^2$ dilution when plating 0.1 ml.

Incubate all plates at 28-30°C for 48 hours and count the number of colonies of different colors.

Count the number of colonies present at each irradiation exposure time and record on the report sheet.

Determine the % kill for each exposure time from the following formula:

$$100 - \left( \frac{\text{Number at end}}{\text{Number at beginning}} \times 100 \right) = \% \text{ kill}$$

Take an isolate of each color of colony and inoculate it into a separate tube of sterile milk medium. Make two sets. Grow one set at 30°C and the other at 37°C. Record any color changes in the color of the milk. Use samples of your control culture (unirradiated culture) for the same test and draw conclusions about the mutagenic effects of ultraviolet light and natural selection pressures.

Make a graph of the number of colonies present compared to the time of irradiation.

## REPORT SHEET FOR EXERCISE 6

**Growth of Ultra Violet Light Irradiated Serratia marcescens**

Colors and Numbers of Colonies After _____ Hours of Incubation

| IRRADIATION TIME | COLONY COLOR | DILUTIONS | | | | | | BEST ESTIMATE OF BATERIA/ML. |
|---|---|---|---|---|---|---|---|---|
| | | $1:10^1$ | $1:10^2$ | $1:10^3$ | $1:10^4$ | $1:10^5$ | $1:10^6$ | |
| Not Irradiated | Red | | | | | | | |
| | White | | | | | | | |
| 30 sec. | Red | | | | | | | |
| | White | | | | | | | |
| 60 sec. | Red | | | | | | | |
| | White | | | | | | | |
| 90 sec. | Red | | | | | | | |
| | White | | | | | | | |
| 120 sec. | Red | | | | | | | |
| | White | | | | | | | |
| 150 sec. | Red | | | | | | | |
| | White | | | | | | | |
| 180 sec. | Red | | | | | | | |
| | White | | | | | | | |

| Irradiation Time (Secs.) | % Killed |
|---|---|
| 0 | _____ |
| 30 | _____ |
| 60 | _____ |
| 90 | _____ |
| 120 | _____ |
| 150 | _____ |
| 180 | _____ |

| ORIGINAL IRRADIATION TIME (SECS.) | STARTING COLOR OF COLONIES | COLOR AFTER INCUBATION FOR _____ HOURS IN MILK MEDIUM AT | | MUTANT ? |
|---|---|---|---|---|
| | | 30°C | 37°C | |
| None | Red | | | |
| | White | | | |
| 30 | Red | | | |
| | White | | | |
| 60 | Red | | | |
| | White | | | |
| 90 | Red | | | |
| | White | | | |
| 120 | Red | | | |
| | White | | | |
| 150 | Red | | | |
| | White | | | |
| 180 | Red | | | |
| | White | | | |

Answer these questions:

1. What conclusions would one draw if white colonies appeared when the cultures were grown at 30°C after irradiation?

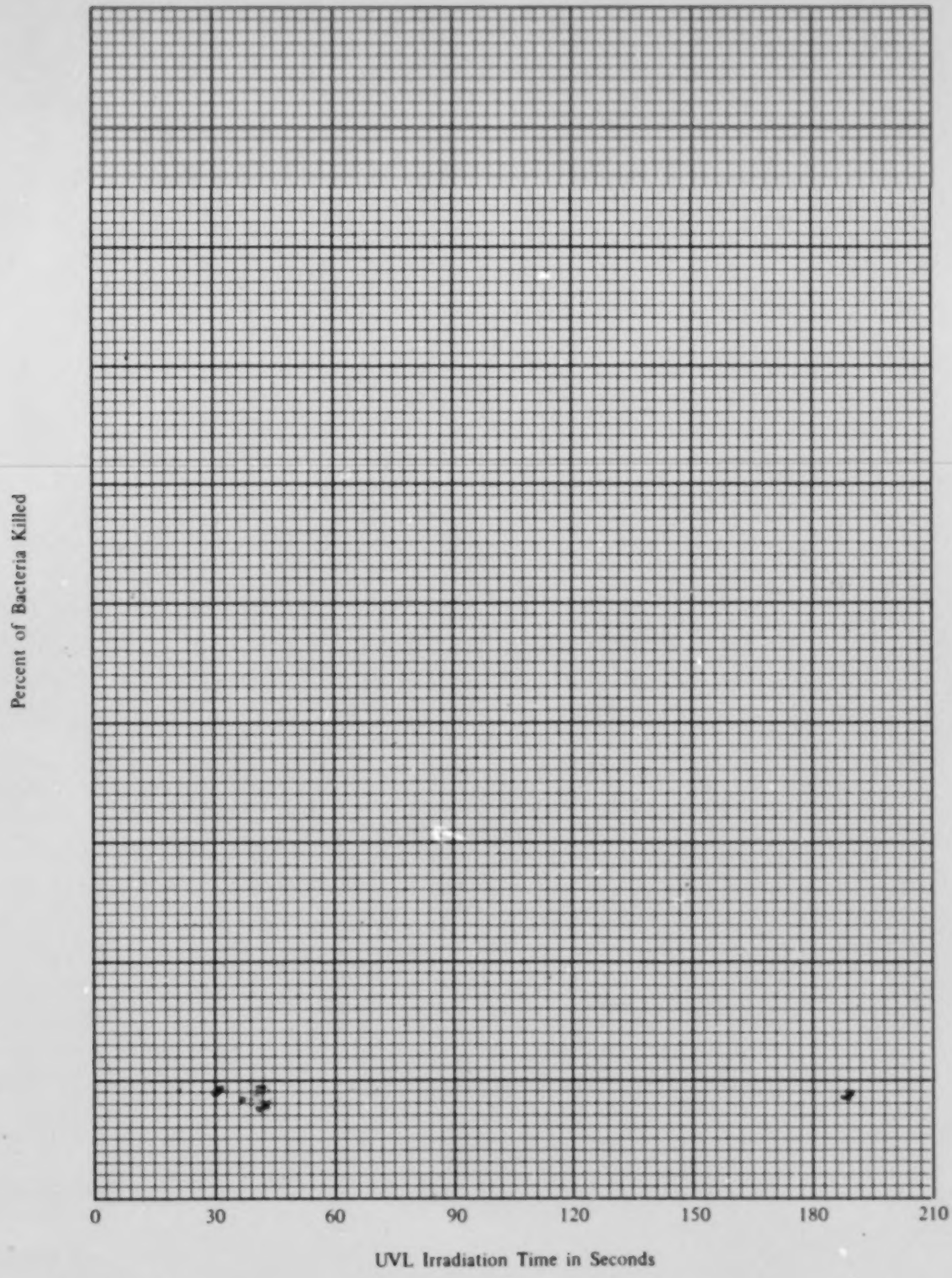
2. What conclusions would one draw if red colonies appeared when the cultures were grown at 30°C after irradiation?

3. If there are other colored colonies present what color are these colonies?

4. Can the mutagenic effects of ultraviolet light be compared with natural selective forces? If so how?

5. Postulate an explanation for the results obtained after irradiation of the bacterial suspension.

## REPORT SHEET FOR EXERCISE 6



## EXERCISE 7 – BUFFERS AND INDICATORS

Materials and Equipment

Per Class

100 ml. of buffers at pHs 3, 4, 5, 6, 7, 8, 9 and 10
50 ml. of these fluids: blood plasma, milk, saliva, urine, egg white, lemon juice, tea, apple juice, carrot juice, carbonated beverage, tap water and distilled water

Per Pair of Students

1 stand with burette holder
2 burettes, 50 ml.
1 100-ml graduated cylinder
4 150-ml beakers
1 glass stirring rod
1 box filter paper
2 funnels
1 wax pencil
1 pHydrion pH paper kit, pH 1-12
1 test tube brush
1 test tube rack, vinyl covered
1 dropping bottle of each of these:
.5% Congo Red, .5% Neutral Red
1.6% Brom Thymol Blue, .05% methylene blue
40 16x150 test tubes
100 ml. of buffer (See Part B, Step 7)

Procedures

**Part A. Titration of .1N NaCl with .1N HCl and .1N NaOH**

1. Mount the burettes upright. Use a piece of filter paper to label them "HCl" and "NaOH." Insert a small funnel into the top of the two burettes. Fill each (as appropriate) with .1N HCl or .1N NaOH. Let some of the fluids run through the tip of burette to get rid of air in the tip and to assure a patent stream of fluid. The meniscus must be located on the burette scale.

2. Measure 40.0 ml. of 0.1N NaCl in a 100-ml. graduated cylinder. Transfer it to a 150-ml. beaker.

3. Take a piece of pHydrion paper about 1.5 inches long. Obtain a drop of NaCl on a stirring rod and determine the pH by dampening the test paper. Repeat for the NaOH and HCl. Record your determination.

4. Now place the beaker under the .1N HCl burette. Add .2 to .3 ml. portions of acid, stir with the rod, and determine the pH with pHydrion paper. Record your results. When 5 ml. have been added in this manner, add 1 ml. portions until 15 ml. have been added. Then add 5 ml. portions three times.

5. Now discard the solution, rinse the beaker, drain, and add another 40 ml. of .1N NaCl to the beaker. Place it under the .1N NaOH burette.

6. Add .2 to .3 ml. portions of alkali, stirring after each addition. Record the pH. When 5 ml. have been added add 1 ml. portions until 15 ml. have been added. Then add 5 ml. portions three times.

**Part B. Titration of a Buffer with .1N HCl and .1N NaOH**

7. Now obtain 40 ml. of .1 M buffer in a clean beaker. One of these will be assigned to your workspace: sodium phosphate, sodium bicarbonate, sodium borate, sodium acetate, glycine, or Tris. Measure the pH.

8. Add 3 ml. portions of 1.N HCl, stirring and taking measurements after each addition until 42 ml. of acid have been added.

9. Wash the beaker and add another 40 ml. of buffer. Repeat step 8 using .1N NaOH.

10. When complete, discard the titration mixture and wash the glassware. Drain.

11. Carry out the graphing of your results and determine the pKa for the buffer by these methods:

Method 1 involves finding the midpoint between the unbuffered lines of the curve. To do this extend a dotted line in the direction of the unbuffered line beyond the point where the line bends. Find the midline between these two. It will intersect the buffered area of the line at the pKa.

Method 2 is Method 1 turned 90°. Draw lines parallel to the abscissa of the graph through the points where the unbuffered part of the graph line ends. The line midway between these should also intersect the titration curve at the pKa.

Method 3 takes advantage of the Henderson-Hasselbalch equation. The change in concentration as one proceeds from the acid to the pKa is logarithmic and again as one proceeds from the pKa to the point where all of the acid has been reacted to form the salt. The amount of the acid form is equal to the amount of standard acid and base needed to proceed from the lowest pH in the buffered area of the titration curve to the highest, and the fraction remaining at any point along the buffered area of the titration curve is equal to the total amount needed minus the amount used to convert acid to salt at that point. These relationships are set forth as follows:

$$pH = pKa + \log([salt]/[acid])$$

so

$$pKa = pH - \log([salt]/[acid])$$

This brings out that when the solution is 10/11 acid it is 1 pH unit below the pKa and when it is 10/11 salt it will be 1 pH unit above the pKa.

**Part C. Pot Pourri**

Because of the way it will be necessary to set up tubes of buffer it will be convenient to make some observations about the color of certain indicator dyes at various pH values as compared with a dye that may be used to indicate something other than pH. At the same time we can also make some observations about the pH at which the isoelectric point is reached for a protein.

1. Label five sets of tubes for each of these pH values and arrange them in your test tube rack(s): pH 3, 4, 5, 6, 7, 8, 9 and 10.

2. Buffer solutions at the above pH values have been prepared and placed on the central supply table. Obtain about 1 inch of the appropriate buffer solution in each tube, that is pH 3 buffer in the tubes labeled 3, pH 4 buffer in the tubes labeled 4, and so forth.

3. At your workspace find dropping bottles containing .5% Congo Red, .5% Neutral Red, 1.6% Brom Thymol Blue, and .05% Methylene Blue. Add 2 to 3 drops of Congo Red to the first series of tubes and record the *color* at each pH. Repeat with the other three dyes. Determine where the pKa

for each dye lies. If it looks like it might lie between two tubes, mix equal amounts from these two tubes and see if an intermediate color is obtained. The pH is in between since all of the buffers are the same strength (.1 molar).

4. To the last set of tubes add dropwise a solution of egg albumin to the pH 5 tube until a persistent white precipitate is obtained. Now add this same amount of egg white to all the other tubes in the set. Let all of the tubes sit for about 15 minutes, then record the amount of precipitate formed as follows:

0 for none, + for light cloudiness, ++ for distinct cloudiness, +++ for a light precipitate and ++++ for a heavy precipitate.

**Part D. The pH of Common Fluids of Biological Interest**

Determine the pH of the following using pHydrion paper strips. Let the strips dry on a piece of paper towelling and then glue it onto your report sheet.

| | | | |
|---|---|---|---|
| Animal Fluids: | 1. Blood plasma | Plant Fluids: | 6. Lemon juice |
| | 2. Milk | | 7. Tea |
| | 3. Saliva | | 8. Apple juice |
| | 4. Urine | | 9. Carrot juice |
| | 5. Egg albumin | | |

Other Fluids: 10. Tap water, 11. Distilled water, 12. Carbonated beverage

Answer the remaining questions on the Report Sheet and turn in before you leave the laboratory.

## REPORT SHEET FOR EXERCISE 7

**Buffers and Indicators**

**Part A. Titration of NaC1 with .1N HC1 and .1N NaOH**

| ML. ADDED | TOTAL ML. ADDED | pH |
|---|---|---|
| | | |

| ML. ADDED | TOTAL ML. ADDED | pH |
|---|---|---|
| | | |

**Part B. Titration of a Buffer with .1N HC1 and .1N NaOH**

BUFFER USED:

| .1N HC1 ML. ADDED | TOTAL ML. ADDED | pH |
|---|---|---|
| | | |

| .1N NaOH ML. ADDED | TOTAL ML. ADDED | pH |
|---|---|---|
| | | |

**Part C. Colors of Dyes at Different pH Values**

| pH | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | — | $I_p$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Dye | | | | | | | | | | |
| Congo Red | | | | | | | | | | |
| Neutral Red | | | | | | | | | | |
| Brom Thymol Blue | | | | | | | | | | |
| Methylene Blue | | | | | | | | | | |

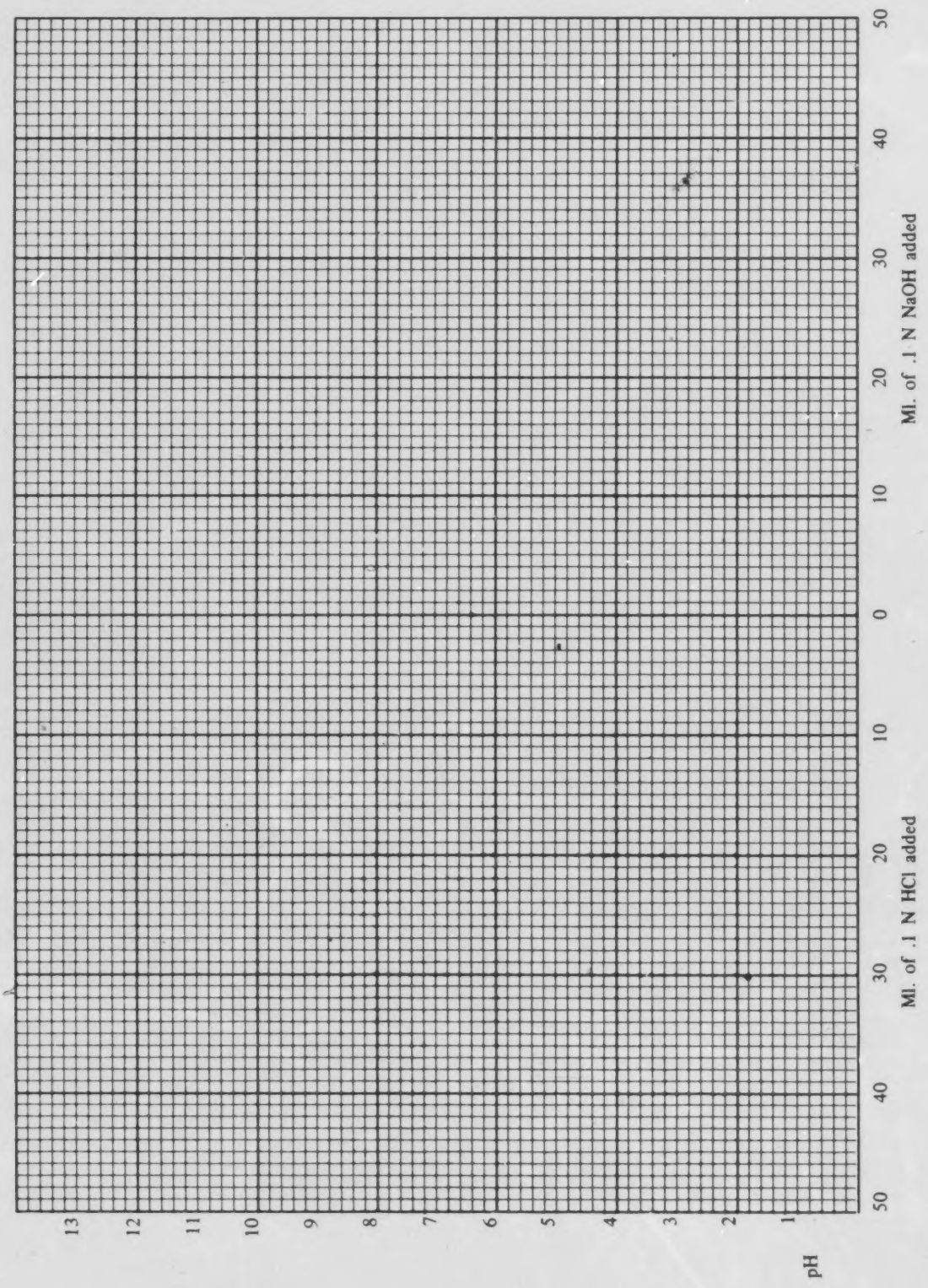
The Iso-Electric Point for Egg Albumin

| pH | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | — | $I_p$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Amount of ppt. formed | | | | | | | | | | |

**Part D. The pH of Common Fluids of Biological Interest**

| FLUID | pH TEST PAPER | pH |
|---|---|---|
| Blood plasma | | |
| Milk | | |
| Saliva | | |
| Urine | | |
| Egg albumin | | |
| Lemon juice | | |
| Tea | | |
| Apple juice | | |
| Carrot juice | | |
| Tap water | | |
| Distilled water | | |
| Carbonated beverage | | |
| | | |
| | | |

## REPORT SHEET FOR EXERCISE 7



## EXERCISE 8 – COACERVATES AND EMULSIONS

Materials and Equipment

Microscope and lamp
Microscope slides and coverglasses
1 10-ml. pipette or 5 ml. pipette
1 medicine dropper
0.1 N Hydrochloric acid
5% gelatin (a protein) in water
5% gum arabic (a polysaccharide) in water
Test tube with stopper and rack
Corn or other vegetable oil
Fat stain such as Sudan III
2% Ca(OH)
Gum acacia
Mortar and pestle
1 100-ml. graduated cylinder

**Part A. Coacervates**

Procedure

1. Mix together in the test tube 5 ml. of 5% gelatin and 3 ml. of 5% gum arabic.

2. Determine the pH of this mixture using pHydrion paper. Place a drop on a microscope slide, cover, and observe under the microscope.

3. Carefully add 0.1N HCl, drop by drop to the test tube. After each addition of acid mix by inverting the tube and then wait a few seconds to see if the mixture becomes cloudy. If the liquid in the tube remains clear, add another drop of acid. Continue adding acid a drop at a time until the mixture becomes cloudy.

4. When the material in the tube turns cloudy, take another pH determination. Then, handling the mixture gently, observe a drop under the microscope for the presence of coacervates. If you do not observe any, decrease the light or go to a higher microscopic power. If you still do not see any coacervates then repeat the whole procedure from the beginning. You may have added the acid too rapidly. When you are successful, record your observations and make sketches of the coacervate droplets.

5. Now add more acid to the test tube, a drop at a time. When the liquid becomes clear again, examine a drop under the microscope and determine the new pH.

Answer the questions on the Report Sheet.

**Part B. Emulsions**

1. Wash a clean mortar and pestle with acetone to remove all traces of grease. Dry.

2. Grind 12.5 grams gum acacia to a powder.

3. Add 50 ml. corn (or other vegetable) oil. Mix about 2-4 seconds to disperse the oil.

4. Add all at once 25 ml. water. Whip rapidly until thick enough to crackle, then dilute with water to 100 ml. (approximately).

5. Remove a small quantity of the emulsion to a watch glass and add a drop or two of Sudan III or Ponceau Red dye–enough to give good color but not too intense. Smear a thin layer on a microscope slide and examine. The dye colors the oil. Which is the dispersed phase, the oil or the water?

6. Add a few drops of fat stain to small quantity of skimmed milk. Make a smear and examine under the microscope. Which is the dispersed phase, the oil or the water?

7. Color a little butter with the fat stain. Examine under the microscope. Which is the dispersed phase, the oil or the water?

8. Color a little soapy solution with fat stain and examine under the microscope.

9. Place some of the emulsion (Step 5) in a beaker and heat over a small flame. Pour into a test tube and let stand until cool.

## REPORT SHEET FOR EXERCISE 8

### Part A. Coacervates

1. Sketch two or three of the coacervates that you saw in the microscope.

2. How do the materials you used in this experiment to make coacervates compare with one that might have been present in the ancient oceans?

3. What is meant by the isoelectric point of a protein and what happens more easily there than elsewhere?

### Part B. Emulsions

| PREPARATION | DISPERSED PHASE |
|---|---|
| Oil emulsion | |
| Skimmed milk | |
| Butter | |
| Soap | |

What happened to heated and cooled oil emulsion?

What is the function of the gum arabic?

What is a colloid?

## EXERCISE 9 – WATER CONTENT OF VARIOUS TISSUES AND CELLS

Materials and Equipment

Per Class

Drying oven
Knives
Bone saws

Per Workbench (4 students)

1 Triple beam balance

Per Student

3 aluminum weighing pans
30-50 grams of one of these: Carrots, potatoes, onions, spinach leaves, dry beans, bean sprouts, yeast cake, liver, kidney, tallow, bone or heart

Procedure

1. Take a pointed but not sharp instrument and impress your initials, section and the number 1, 2 or 3. Do not use a pencil or pen as the weight of the marking substance will rub off and change the weight of the pan.

2. Weigh each pan as accurately as you can and record the weights.

3. You will be assigned an animal, plant or microbial cell preparation by the teacher. Place 10 to 20 grams of the material in the weighed pans and reweigh. Record the gross weights.

4. Place the pans in the drying oven set for 100–110°C to dry. Materials will dry more quickly if they are cut into small pieces.

5. When your material has dried to a constant weight, reweigh all the pans. Subtract the tare weights and determine the net dry weights.

6. Compute the per cent water by dividing the wet weight of each tissue into the dry weight and multiplying by 100.

7. Compute the mean and standard error for your data. One method is given below:

| % Water | Deviation from Mean | $(\text{Deviation})^2$ | |
|---|---|---|---|
| 77 | 2 | 4 | n = number of pans, in this case, 3 |
| 73 | -2 | 4 | |
| 75 | 0 | 0 | |
| 225 Sum | 0 | 8 = Sum of deviations squared $S(d)^2$ | |
| 75 Mean | | | |

4.0 = Variance = $S(d)^2$ /(number pans minus 1)
1.33 = (Standard deviation of the mean)$^2$ = $s^2/n$
1.15 = Standard Error = square root of $s^2/n$

Therefore the mean ± S.E. = 75 ± 1.15

The standard error estimates the range from the sample mean within which the mean for a large number of samples should fall.

## REPORT SHEET FOR EXERCISE 9

### The Water Content of Various Tissues

What material did you use? _____

| | Pan 1 | Pan 2 | Pan 3 |
|---|---|---|---|
| Gross Wet Weight | | | |
| Tare | | | |
| Net Wet Weight | | | |
| Gross Dry Weight | | | |
| Tare | | | |
| Net Dry Weight | | | |
| % Water | | | |

Computation of the Mean and Standard Error

| Pan | % Water | Deviation (d) | $d^2$ |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| Sum | | | |
| Mean | | | |

_____ = Standard deviation squared

_____ = Standard error

Questions

1. Why do tissues contain so much water as compared with other components?
2. What is meant by bound and free water?
3. Why do solutes come out of water solutions when they freeze?

## REPORT SHEET FOR EXERCISE 9

**Class Results for the Water Contents of Various Tissues and Cells**

| MATERIAL | NUMBER OF PANS | WATER CONTENT (MEAN ± STD. ERROR) |
|---|---|---|
| | | |

## EXERCISE 10 – CELL TYPES

It has been a frequent custom in the past to draw a diagram of a cell which would include as many features as desired from the study of a variety of cells at different magnifications, and then label this a "typical cell." Of course, such a cell diagram would hardly be typical. There are instead a number of cell varieties, each one typical of the variations on that type. Yet, they all seem to hold a number of structures in common. We shall look at several types of cells in this exercise. The student should study each one and then reach some generalizations (conclusions) about what is typical of living and preserved cells.

### Materials and Equipment

Microscope and lamp
Microscope slides and coverglasses (coverslips)
Petrolatum (Vaseline) gun (a 2 cc. syringe without needle)
Melted petrolatum and small camel hair brush
Syracuse watch glass
Medicine dropper
125 mg% Neutral Red in 95% alcohol
100 mg% Janus Green B in 95% alcohol
Forceps, scissors, dissecting needle and single-edged razor blade
5% methyl cellulose

Living cultures: Fresh onions, Elodea sprigs, Nitella, baker's yeast, Escherichia coli, Pelomyxa or Chaos chaos (Amoeba), Stentor

Prepared slides: Mammalian liver stained with haematoxylin and eosin, mitochondria in liver cells, Golgi complex, leaf types, sea urchin eggs, bull sperm, Ascaris megalocephala sperm entrance

### Slide Preparations

Two precautions in making temporary mounts: 1–The coverglass must be supported a slight distance above the specimen so as not to mash it. 2–The specimen must be kept wet. This may be done by adding more fluid to the edge of the coverglass or by sealing the fluid under the coverglass by using a petrolatum barrier to prevent evaporation.

### Simple wet mount

Lay down a spiral of petrolatum about the size of a dime using the petrolatum gun. The opening of the spiral permits excess fluid to escape as the coverglass is pushed close to the slide. The specimen is transferred to the spiral in a small drop of fluid and coverslipped.

### Open-ended wet mount

Lay down two parallel lines of petrolatum the length of the coverglass. Transfer the specimen to the included space with a dropper to Coverslip. Fluids beneath the coverglass can then be exchanged by applying a drop to one side, and removing excess fluid with a bit of paper towelling (or bibulous paper) from the opposite side. Fluids must be replaced often to prevent the specimen from drying out.

Either simple wet mounts or open-ended wet mounts may be kept longer (for hours) by sealing around the edges with melted petrolatum applied with a small camel hair brush.

Hanging drop preparation

If the specimen would normally float, then a hanging drop preparation is in order for upright microscopes. The specimen is dropped onto the coverglass. The surface around the excavation on a depression slide is greased lightly with petrolatum and then placed over the coverglass on the table top. The whole assembly is lifted and turned over quickly so as to not allow the drop to run. Allow a few seconds for the specimen to rise against the coverglass. (Note: If the specimen drop runs, start over.)

Supravital Staining

Vital staining is a process whereby living cells and tissues are stained in the animal body by dyes that do not significantly interfere with their vital processes. Supravital staining involves the staining of cells and tissues that have been removed from the body and stained while yet alive. The most commonly used vital dyes include Nile Blue A (or sulfate), Bismarck Brown, Janus Green B, Neutral Red and Methylene Blue.

The following procedure permits the staining of cells in their undiluted natural medium. This is accomplished by the application of an alcoholic solution of the dyes to a slide and then letting the alcohol evaporate. To do this:

1. Wash several microscope slides in detergent. Rinse thoroughly in tap and distilled waters and finally dry them from 95% alcohol with a lint-free cloth. Handle the slides with forceps or by the edges. Flame them over a bunsen burner to remove any traces of lint and grease and store in a dust-free, covered box.

2. Stock Solutions: Dissolve 125 mg. Neutral Red in 50 ml. 95% alcohol. Dissolve 100 mg. Janus Green B in 50 ml. 95% alcohol.

For use: Mix 8 ml. of the Neutral Red solution with 42 ml. 95% alcohol then add 8 ml. of the Janus Green B stock. Use immediately as the dyes will precipitate on standing for a short time.

Flood the cleaned, dry slides with the stain mixture. Drain quickly. Dye films prepared on a humid day will not be as even as those made on dry days. The slides will keep indefinitely if protected from dust.

Sketches and Drawings

1. Place sketches and drawings *above* the legends provided.
2. Place drawings to the left and label neatly to the right side.
3. Make drawings and sketches large enough to show details easily. Most drawings should be at least 1½ inches across.

Plant Cells

1. Onion Epidermal Cells. Remove a fresh scale from an onion and with razor blade and forceps remove the inner epidermal layer. Transfer to water in a Syracuse watch glass. Cut into suitable pieces with sharp scissors and transfer to an open-ended wet mount. Observe unstained with low illumination. You should be able to see the cell wall, central vacuole and perhaps the thin layer of cytoplasm adjacent to the cell wall. Now draw some acetocarmine or methylene blue stain under the coverslip followed by several drops of tap water to wash away the excess stain. You should now be able to see the nucleus. Sketch one cell and label the cell wall, cytoplasm, nucleus and vacuole.

2. Streaming in Nitella cytoplasm. Make a simple wet mount of a few filaments of the alga Nitella. Observe under high dry magnification with dim illumination. Is there any movement to be seen in these cells? Diagram a cell, labeling its parts, and indicate the direction of any movement observed in the cytoplasm.

3. Streaming in Elodea. Choose a young leaf near the end of a sprig of Elodea (the water weed). Remove it with forceps, being careful to leave the tip of the leaf unbruised. Make a simple wet mount and observe under the microscope with bright illumination. Sketch two or three contiguous cells and indicate in which directions movement is observed. Label the vacuole, cytoplasm, chloroplasts and the cell wall.

4. Palisade cell from a green leaf. In your slide collection find the slide labeled "Leaf Types." Study and compare the structure of these three types of leaves. Sketch a palisade cell from a dicot leaf. Label the nucleus, chloroplasts, cytoplasm and vacuole.

Microbial Cells

5. Yeast Cells. To a drop of active yeast culture on a microscope slide add a drop of .05% methylene blue. Add a coverslip and seal with petrolatum. Sketch a cell and label the features demonstrated.

6. E. coli. Flame and cool a nichrome wire loop and transfer a loopful of E. coli culture to a clean microscope slide. Spread the drop and let it dry in air. Fix the slide by passing the slide, cell side up, through a small flame two or three times (until just warm to the back of the hand). Stain by applying a drop of Hucker's crystal violet for about 30 seconds. Rinse with a gentle stream of tap water and let dry in air. Clear the preparation with a drop of immersion oil. Find the field under low power, then high-dry power. When you have selected a good area of the slide for study, one not too crowded with cells, go to oil immersion. Sketch two or three cells.

Animal Cells

7. Pelomyxa or Chaos chaos (a giant amoeba). These organisms appear as white specks on the bottom of the culture dish. Use a medicine dropper to aspirate one or more and transfer them to a Syracuse watch glass. Obtain a slide that has been treated with Neutral Red as described on page 4-2. Prepare a simple wet mount and transfer the cell to the stained slide. From your knowledge of the colors of Neutral Red in acid, neutral and alkaline media estimate the pH in various components of the cell. Note also the way in which amoeba moves. S. O. Mast has described the components of amoeboid movement as follows:

Movement of the plasmasol toward a pseudopod (anteriad)
Gelation anteriad
Solation posteriad with a slight contraction
Movement of the plasmalemma over the whole

Can you verify Mast's observations? How can the movement of the amoeba be explained?

Make a sketch of the amoeba indicating the following: The plasmalemma, the plasmagel, the plasma sol, pseudopods. Indicate with arrows the direction in which the cytoplasm is moving.

Set the slide aside and look at it again in about 10 minutes and again in about 20 minutes. Where is the Neutral Red dye located in the cell? How can this be explained?

8. Stentor. Prepare a simple wet mount. Place a small drop of 5% methyl cellulose in the center of the slide and transfer onto that a specimen of Stentor coereleus. Note the coordination of the ciliary movement. Tap the slide gently. Does this animal contract? Can you see any longitudinal filaments called myonemes? The blue-green color comes from the color of numerous algal cells that embedded themselves in the wall of Stentor. These are commensals. What benefits does Stentor derive from their being there? What benefit do the algal cells derive from being there?

9. An Animal Egg Cell. Find a slide of Ascaris eggs (sperm entrance). These are probably the most idealized of the cells we will look at today. They are protected by a gelatinous coat. The cell limit is visible. The eccentric nucleus has a distinct nucleolus or two. Since this is a microlecithal egg the yolk granules may be difficult to see. However, cytoplasm containing yolk is called *deutoplasm*. Draw an egg. Label all of its parts.

10. A Sperm Cell. Find the slide labeled "Bull Sperm." These are rather typical mammalian sperm cells. Study them under oil immersion and identify the acrosome (Golgi material), the head (nuclear material), the ring centriole, the midpiece which contains many mitochondria, and the tail filament which is a long flagellum arising from a centriole.

Did you see a centriole in the egg cell? Look again.
Find the slide labeled Ascaris megalocephala, sperm entrance. Can you identify the sperm cell of Ascaris? How is it different from bull sperm? Does it have a centriole? Do Ascaris eggs have a centriole? Find an example where the sperm has entered an egg. Does such an egg have centrioles?

11. Look at a slide of mammalian liver. There are many similar cells in a tissue like this. Make a large drawing of a single cell. Label the cell limit, the cytoplasm, the nucleus, and any other features you can identify.

12. Find the slide labeled "Mitochondria." Make a drawing of similar size to that in 11, indicating the locale of the mitochondria and their shape.

13. Golgi Apparatus. This controversial cell organelle is located near the nucleus. Sketch the cell and draw in the Golgi material.

## REPORT SHEET FOR EXERCISE 10

**Cell Types**

1. Onion Epidermal Cells taken from the inner surface of an onion scale and stained with _____ _____. Drawn from _____X.

2. Nitella Cell. The arrows indicate the direction of movement observed. Drawn from _____X.

3. Elodea Leaf Cells. Arrows indicate the direction of movement observed. Drawn from _____X.

4. Palisade Cell from the mesophyll layer of a green leaf. Drawn from _____X.

5. Yeast Cells stained with methylene blue. Drawn from _____X.

6. E. coli stained with Hucker's crystal violet. Drawn from _____X.

7a. Pelomyxa (Chaos chaos) supravitally stained with Neutral Red
Arrows indicate the direction of movements seen in the cell.
Drawn from _____X.

7b. Pelomyxa _____ minutes after being stained with Neutral Red.
Drawn from _____X.

8. Stentor, a free-living ciliate.
Drawn from _____X.

9. Egg cell of _____
Drawn from _____X.

10. Bull spermatozoan cell. Drawn from _____X.

11. Parenchymal cells from the mammalian liver. Fixed and stained with haematoxylin and eosin. Drawn from _____X.

12. Mitochondria demonstrated in the liver of the _____ using the technique of Bensley. Drawn from _____X.

13. Golgi Apparatus demonstrated in a _____ cell of the _____ Drawn from _____X.

**Cell Types**

Questions

1. State the Cell Theory.
2. What are the implications of the Cell Theory?
3. What is our course description of a cell? Do all cells studied today conform with this description?
4. Bull spermatozoa do not divide and they have an "odd" shape. What arguments can be made for calling them cells within our course definition?
5. Were you able to see a centriole in an unfertilized Ascaris egg? In a fertilized egg? Where did it come from?
6. How can one explain the streaming of the cytoplasm observed in Nitella?
7. How can the form of proteins be related to movement in amoeba? Would the location of Neutral Red staining after several minutes back up this view?

# EXERCISE 11 – TYPES OF FOODS FOUND IN CELLS AND TISSUES

Materials and Equipment

Boiling water bath (heater or burner and beaker)
36 test tubes and rack
1% glucose
1% starch
1% egg albumin
1% glycine
Corn oil
Irish potato
Turnip
Peanuts
Liver
Chicken breast muscle
Mortar and pestle
Sea sand (fired)
Flask, 250 ml.
Magnesium sulfate
Petroleum ether
Acetone
Small funnel and filterpaper
Lugol's iodine solution
Benedict's Quantitative solution
.25% Ninhydrin in 1 M Phosphate buffer
Biuret reagent

**Part A. Test for Various Foods**

Procedure

Set up the water bath and start the water to heating.
Number the tubes appropriately with a wax pencil, marking near the tops.
Ascertain where the things are that you need to work with before you begin.
Arrange test tubes in your test tube rack.

1. Place about 1 ml. of 1% glucose in four tubes numbered 1, 11, 21, 31.
   Place about 1 ml. of 1% starch in four tubes numbered 2, 12, 22, 32.
   Do the same for 1% egg albumin (tubes 3, 13, 23, 33), for 1% glycine (tubes 4, 14, 24, and 34) and corn oil (tubes 5, 15, 25 and 35).

2. Take tubes 1, 2, 3, 4 and 5.
   Add 3 drops of Lugol's iodine solution (1% iodine in 1% KI).
   Record the colors produced on the report sheet.
   Which of these reactions might be an identification test?

3. Take tubes 11, 12, 13, 14 and 15.
   Add an equal volume of Benedict's solution.
   Place in the boiling water bath for 3 minutes.
   Record the colors observed.
   Which of these reactions might be an identification test?

4. Take tubes 21, 22, 23, 24 and 25.
   Add 1 ml. of .25% ninhydrin solution buffered at pH 7.
   Heat in boiling water for 10 minutes.
   Cool. Add 5 ml. of 95% alcohol to each tube.
   Which of these reactions might be an identification test?

5. Take tubes 31, 32, 33, 34 and 35.
   To tube 33 add biuret reagent dropwise until a definite color develops.
   Add the same amount of biuret reagent dropwise to tubes 31, 32, 34 and 35.
   Record the reactions.
   Which of these reactions might be an identification test?

6. Rub each substance into a clean piece of paper. Fats will produce a singular effect.

**Part B. Foods in Cells and Tissues**

1. Cut plugs of white potato with a size 1, 2 or 3 cork borer.
   Place the plugs in tubes numbered 6, 16, 26 and 36.
   Place plugs of turnip in tubes numbered 7, 17, 27 and 37.
   Place a skinned peanut in tubes 8, 18, 28 and 38.
   Place a small piece of chicken breast muscle in tubes 9, 19, 29 and 39.
   Place a piece of liver about the size of a pea in tubes 10, 20, 30 and 40.

2. Take tubes 6, 7, 8, 9 and 10. Add three drops of iodine.
   Record your observations.

3. Take tubes 16, 17, 18, 19 and 20.
   Add 1 ml. of Benedict's solution.
   Place in the boiling water bath for 3 minutes.
   Record your observations.

4. Take tubes 26, 27, 28, 29 and 30.
   Add 1 ml. of .25% ninhydrin solution buffered at pH 7.
   Place in the boiling water bath for 10 minutes.
   Cool. Add 5 ml. of 95% alcohol.
   Record your observations.

5. Take tubes 36, 37, 38, 39 and 40.
   Add the same amount of biuret reagent dropwise used in Part A5.
   Record the reactions.

6. Extraction of lipid (fat).
   Grind about 1 gram of potato with sea sand in a mortar with the pestle.
   Add about an equal amount of magnesium sulfate to absorb water.
   Grind well, into a paste.
   Add 5 ml. of acetone and 5 ml. of petroleum ether (measured in a graduate).
   Make a good slurry. Filter.
   Evaporate the filtrate in the hood or in a well ventilated place over a boiling water bath (making use of a hot plate). Keep the acetone and ether at least 25 feet away from flames in a well-ventilated room.

   Repeat for each of the other foods being tested.

   When the residue is obtained from evaporation, rub some of it into clean paper.
   Rate the various substances as +++ (very oily), ++ (slightly oily), + (a trace of oil) or 0 (no oil).

## REPORT SHEET FOR EXERCISE 11

**Foods Stored in Cells and Tissues**

| PART A | LUGOL'S IODINE | BENEDICT'S TEST | NINHY-DRIN TEST | BIURET TEST | PAPER TEST |
|---|---|---|---|---|---|
| 1% Glucose | | | | | |
| 1% Starch | | | | | |
| 1% Egg albumin | | | | | |
| 1% Glycine | | | | | |
| 1% Peanut oil | | | | | |
| PART B | | | | | |
| Irish Potato | | | | | |
| Turnip | | | | | |
| Peanut | | | | | |
| Liver | | | | | |
| Chicken Breast Muscle | | | | | |

In Part A of the chart circle those reactions which are identifications.

Questions

1. In what forms will foods be stored in cells and tissues?

2. What is the more usual name for "animal starch"?

3. What effect will feeding much more carbohydrate than can be used for energy needs have on the composition of food stored in cells?

# EXERCISE 12 – DIFFUSION, OSMOSIS AND ACTIVE TRANSPORT

## Part A. Diffusion of Hydrogen and Hydroxyl Ions Through Gelatin

1. Cut a 12-inch length of ¼-inch cellophane dialysis tubing and soak it in a beaker of water for a few minutes until it becomes soft. Work open the ends, insert a small funnel, and fill with tap water. Hold the ends and squeeze the water toward the center of the loop. If there are any holes in the tubing a fine stream of water will be expelled. Such a piece of tubing must be replaced.

2. To a hole-free piece of tubing add about 6 inches of melted 10% gelatin containing enough bromthymol blue (0.016%) to give it a definite color. Center the gelatin in the tubing and insert into a beaker containing .1N HCl. Note the time you put the tube into the acid and the time until it has all turned one color. What is the color?

3. Remove the bag of gelatin from the acid. Rinse it off in tap water and transfer it to a beaker of .1N NaOH. Note the time and the approximate time for it all to change color. What would a green band indicate? What is the final color?

4. Answer the questions for the section on the Report Sheet.

## Part B. Osmosis

1. Obtain 7 test tubes and mark them 0%, 1%, 2%, 3%, 4%, 5% and 10%. Fill them about 3/4 full of the corresponding concentration of NaCl solution.

2. Cut six plugs of white potato with a cork borer. Size *1-3* is suggested. The plug must fit easily into the 10 ml. graduated cylinders you will use to measure the volume of each plug.

3. Measure the volume (using tap water) and weigh each plug, then transfer it to a tube of NaCl. Record the weight, volume, tube number and starting time on your Report Sheet.

4. After 90 to 120 minutes recover the potato plugs from each tube (one at a time so that they do not get mixed up.) Blot away the excess solution with a damp (not wet) piece of paper towelling and determine its weight and volume again. How does each plug feel? Record your data. Return the plugs from 0% and 10% to their tubes of NaCl for use in the next section of this exercise. Discard the others.

5. Plot the data for volume and weight on the graphs provided (Graphs 1 and 2).

6. Plot the data for percent change in weight against the reciprocal of the NaCl concentration (Graph 3). Do the same for the percent change in volume (Graph 4).

**Part C. Plasmolysis in Elodea Leaf**

1. Make an open-ended wet mount of an Elodea leaf so that fluids can be exchanged under the coverglass (see Exercise 10).

2. Find a green cell and sketch it.

3. Draw a 10% NaCl solution under the coverglass, drawing water off the other side with a bit of paper towelling.

4. Sketch the same cell at 1 minute intervals for 5 minutes. What happens to the vacuole? The plasma membrane? The cell wall?

5. Now add water to one side of the slide and draw off the salt solution. Wash the leaf well in this way. Now sketch the cell at 1 minute intervals for 5 minutes.

6. Take a single-edged razor blade and cut a thin wedge from the potato plug in water (0% NaCl). Add a drop of Lugol's iodine solution to it in a Syracuse watch glass. Drain and rinse with water. Make a wet mount and observe under the microscope. The iodine will have stained the starch grains dark blue or black. Identify the cell wall, vacuole and the starch grains. Sketch. Repeat this procedure with the potato plug in 10% NaCl, remembering to use 10% NaCl to rinse away the Lugol's solution. Sketch a cell and label as above.

7. Answer the questions on this section in the Report.

**Part D. Active Exlusion of a Substance**

1. Label 3 test tubes: N (normal), B (boiled) and P (poisoned).

2. Add about 5 ml. of a growing yeast suspension to each.

3. Place tube B in a container of boiling water or heat over a bunsen burner until the tube contents boil for at least 15 seconds. This will kill the cells.

4. Add 5 drops of 0.01 M KCN and 5 drops of 1 M iodoacetate to the tube labeled P. (REMEMBER: Both KCN and iodoacetate are poison. If you get any on your hands, wash them immediately.)

5. Now to each tube add enough .5% Congo Red to give a good color. Record the color in each tube on your report sheet.

6. Examine a drop of each suspension under the microscope starting with tube N. What colors are the cells in each tube? Are all of those in tube N stained? How about tubes B and P?

7. Place a small funnel into each of three clean test tubes. Prepare a filter paper for each. Filter the remaining contents of each tube. Record the color of the cell on the filter paper and the filtrate that comes through.

Turn in your Report before you leave for the day.

## REPORT SHEET FOR EXERCISE 12

**Diffusion, Osmosis and Active Transport**

**Part A. Diffusion of Hydrogen and Hydroxyl Ions Through Gelatin**

Starting color: _____ Color in .1N HCl: _____
Time into the acid _____ Time color complete _____ Minutes _____
Radius of the tubing is _____ inches or _____ cm. or _____ mm.
What is the approximate diffusion rate? _____ mm./sec. or _____ cm./min.
Color in alkali _____
Time into .1N NaOH _____ Time color complete _____ Minutes _____
Approximate diffusion rate is _____ mm./sec. or _____ cm./min.

Did you observe a green band?
What did it indicate?

What caused the hydrogen ions to move into the gelatin?

According to the kinetic theory, in what directions are the ions moving?

What is meant by the flux of an ion?

In what ways does a cellophane (cellulose acetate) membrane differ in its properties from a plasma membrane?

**Part B. Osmosis**

Why does the curve have the shape it does in Graphs 1 and 2?

## Part B. Osmosis

| Conc. of NaCl: | TIME EXPOSED TO NaCl: _____ MINUTES 0% | 1% | 2% | 3% | 4% | 5% | 10% |
|---|---|---|---|---|---|---|---|
| Starting weight of potato plug in mg. | | | | | | | |
| Final weight of potato plug in mg. | | | | | | | |
| Change in wt. (mg.) | | | | | | | |
| % Change in weight | | | | | | | |
| Starting volume of potato plug in cc. | | | | | | | |
| Final volume of potato plug in cc. | | | | | | | |
| Change in vol. (cc.) | | | | | | | |
| % Change in volume | | | | | | | |
| How does the plug feel at the end of the experiment? | | | | | | | |

## Part C. Plasmolysis

Questions

1. Plasmolysis means literally to dissolve the cytoplasm. Did that occur?

2. How did the cell wall respond to NaCl?

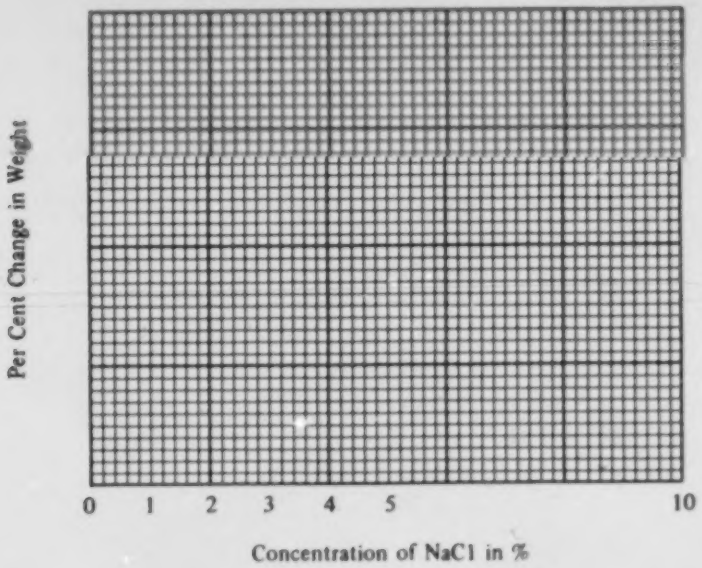
3. What response or what behavior was exhibited by the plasma membrane (cell limit)?

4. Why did the vacuolar contents behave as they did?

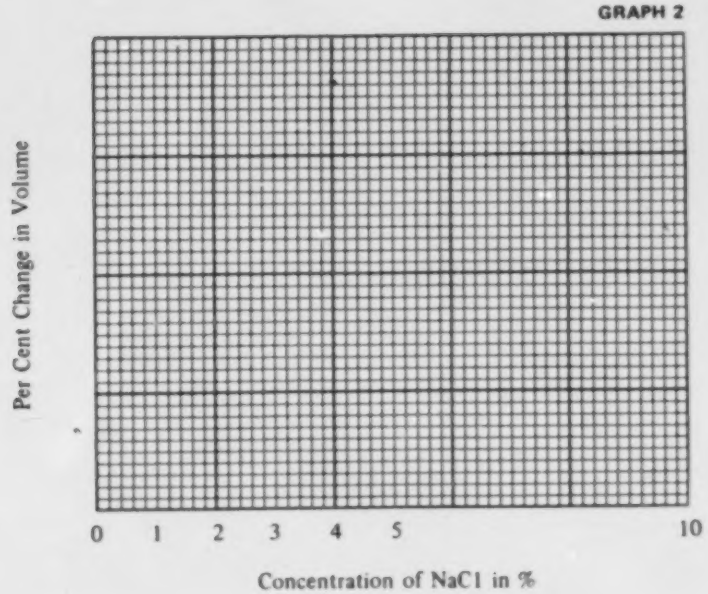
## REPORT SHEET FOR EXERCISE 12

Osmosis

GRAPH 1



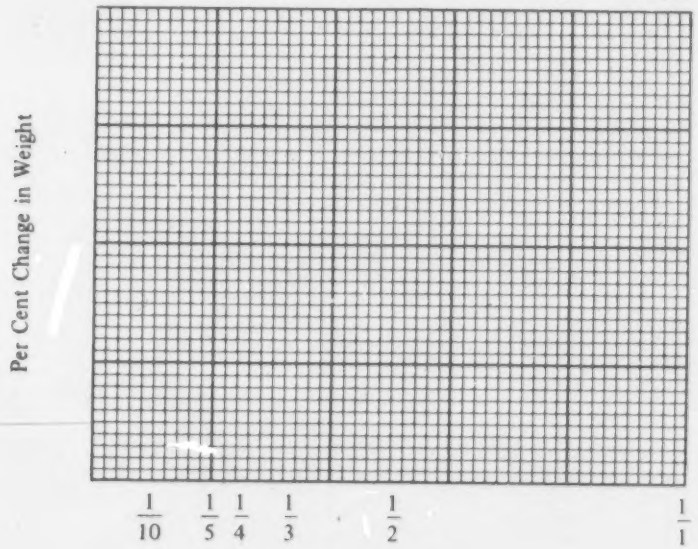

GRAPH 2



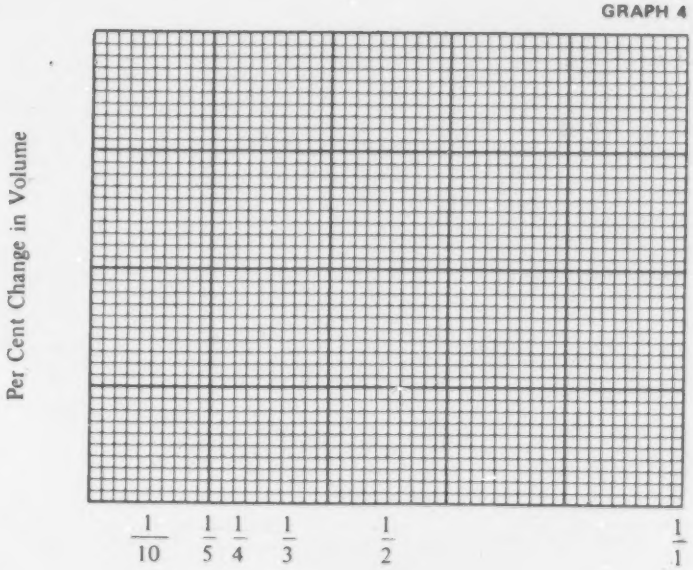
## REPORT SHEET FOR EXERCISE 12

Osmosis

GRAPH 3




GRAPH 4



## REPORT SHEET FOR EXERCISE 12

### Active Exclusion of a Substance

| | TUBE N | TUBE B | TUBE P |
|---|---|---|---|
| Percent of cells in the field that are stained | | | |
| Color of cells stained under the microscope | | | |
| Color of the tube contents | | | |
| Color of the filtrate | | | |
| Color of cells on the filter paper | | | |

What color changes did you find with change in pH for Congo Red in Experiment 3?

How do you explain the number of cells stained in Tube N?

How do you explain the number of cells stained in Tube B?

How do you explain the number of cells stained in Tube P?

Would you conclude from your observations that Congo Red can pass through the cell membranes of yeast? Why?

Did the cell membrane protect the cell from a harmful substance (KCN)?

What is the difference in the mechanisms operating in Tubes B and P?

## REPORT SHEET FOR EXERCISE 12

**Plasmolysis in Elodea Leaf**

START

Time after adding 10% NaCl.

1 MIN. 2 MIN. 3 MIN. 4 MIN. 5 MIN.

Time after washing with distilled water.

1 MIN. 2 MIN. 3 MIN. 4 MIN. 5 MIN.

Sketch of cell from potato plug kept in 0% NaCl and stained with Lugol's iodine solution.

Sketch of cell from potato plug kept in 10% NaCl and stained with Lugol's iodine solution.

## EXERCISE 13 – ACTIVITIES OF ENZYMES

Materials and Equipment for Parts A and B

8 Test tubes and rack
Wax pencil
Mortar and Pestle
Sea sand (fired)
Manganese dioxide
Hydrogen peroxide, 3%
Tincture Gum Guaiac in dropping bottle
Splints
Liver from freshly killed and bled animal

Procedure

1. Mark two test tubes 1 and 2. Into No. 1 add about 2 cm. of water. Put about 2 cm. of hydrogen peroxide into tube No. 2.

2. To each tube add a pinch (about .1 gm.) of manganese dioxide ($MnO_2$ ) and shake to mix.

3. Are any bubbles generated? Take a glowing splint and insert it into tube No. 1, then into tube No. 2.

4. Record your observations.

**Part B. An Enzyme in Liver**

Catalase is an enzyme that is abundant in the liver.

1. Label five test tubes 1, 2, 3, 4 and 5.

2. Obtain a piece of fresh liver about the size of a green pea and place it in tube No. 1.

3. Obtain a piece of fresh liver about the size of 2 or 3 peas and place it in a clean mortar. Add a little fired sea sand and grind the tissue to a paste with the pestle. Add about 5 ml. of .9% NaCl, mix well, and pour the suspension into a beaker. Such a ground bit of tissue is called a homogenate if the cells are broken up, and sometimes a *brei*.

Add 1 ml. of the brei to tubes 2, 3 and 4. Heat tube No. 4 over a flame until it boils for about 15 seconds. Cool.

4. To tube No. 5 add some fired sea sand to about a depth of .5 to 1 cm.

5. Add 5 drops of tincture gum guaiac to each test tube.

6. Add about 1 cm. of water to tube No. 3 and about 1 cm. of hydrogen peroxide to tubes Nos. 2, 4 and 5. Use the glowing splint test on all tubes to see if the gas generated will support combustion.

**Part C. Effect of pH, Concentration and Temperature on the Rate of Enzyme Reactions**

This experiment may be done as a demonstration or as a special project by two or more students.

Material and Equipment

B&L Spectronic 20 or 340 Spectrophotometer
2 B&L Spectronic 20 tubes
Water baths at 27°, 37° and 57°C.
Alkaline Phosphatase, .5% in double-distilled water
Acid Phosphatase, .5% in double-distilled water and .2% para-Nitrophenyl phosphate
0.1 M buffers as follows: HAc-NaAc at pH 3 and 5.5; Tris-HCl at pH 7.0 and 8.5 and Glycine at pH 10.0 and 12.0
Test tube rack and 30 test tubes
Wax pencil
0.1N NaOH
1 Stop clock

**Experiment 1–Effect of pH on Alkaline and Acid Phosphatase**

Consideration of an Hypothesis. Do these proteins behave the same at all pH values? Write an hypothesis for this experiment on your report sheet.

Design of a Controlled Experiment. A control group or tube is needed in order to know what would have happened if the experimental procedure had not been carried out on the material under study. In this case it is called a "blank," that is, at least one tube is prepared with all of the components under circumstances where no reaction occurs so that the color generated by the components can be negated when the blank is set for ZERO optical density (O.D.) in the spectrophotometer. See directions at the end of this exercise for operation of the B&L Spectronic 20 or 340.

Procedure

1. Mark two sets of 6 test tubes for pH 3, 5.5, 7, 8.5, 10 and 12. Mark one set Alk. and the other Acid. Mark a tube "Alk. Blank" and another "Acid Blank." Add 1 ml. of pH 5.5 buffer to the Acid Blank and 1 ml. of pH 10 buffer to the other.

2. Add 1 ml. of p-nitrophenylphosphate solution to each tube.

3. Add 1 ml. of .5% alkaline phosphatase to the "Alk." series of tubes and 1 ml. of .5% acid phosphatase to the "Acid" series of tubes. Do not add enzyme to the two blanks. Start the timer. Record the temperature.

4. At the end of 30 minutes add 4.0 ml. of .1N NaOH to each tube in the acid series and 10 ml. of .1N NaOH to each tube in the alkaline series. Proceed in the same order as in adding the enzyme. Add 4 ml. of NaOH to the Acid Blank and 10 ml. to the Alkaline Blank. Now add 1 ml. of each enzyme to the proper blank.

5. Read the color developed in the spectrophotometer, setting the O.D. to ZERO with the Acid Blank for the acid phosphatase series and setting to ZERO with the Alkaline Blank for the alkaline phosphatase series. The amount of color that is developed is directly proportional to the amount of para-nitrophenol released by the enzymes.

6. Record your O.D. readings and plot them on Graph 1.

**Experiment 2–Effect of Temperature and Concentration On Reaction Rate**

Consideration of an Hypothesis. What effect will temperature and concentration have on the rate of reaction? Write an hypothesis for this experiment on your Report Sheet.

Procedure

1. Mark 5 test tubes for each of these temperatures: 1°, 27°, 37° and 57°. Mark two tubes at each temperature "0.2%" and two "0.5%." Mark the 5th one "Blank."

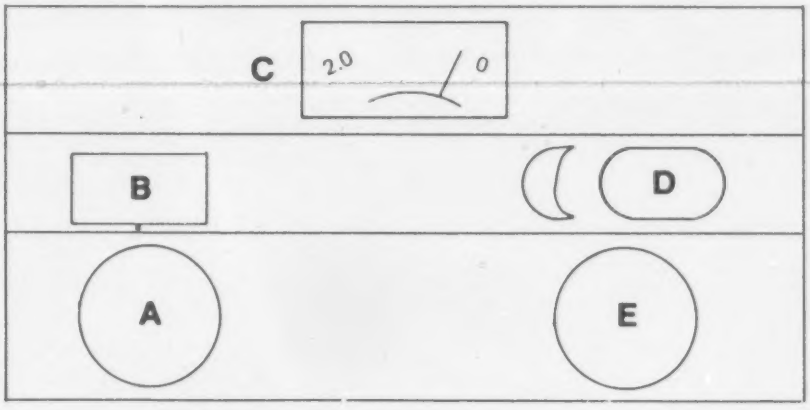
2. Add 1 ml. of pH 5.5 buffer and 1 ml. of p-nitrophenylphosphate solution. Then add 1 ml. of acid phosphatase to each tube except the blanks.

3. Place the 1° tubes in the cold room and the others in water baths at the indicated temperatures. Start the timer.

4. At the end of 30 minutes stop the reactions by adding 4.0 ml. of .1N NaOH to each tube including the blanks. Then add 1 ml. of acid phosphatase (.5%) to each blank.

5. Set the O.D. to ZERO with the blank for each temperature group. Read the O.D. at 405 millimicrons and record your data on the Report Sheet. Plot your data on Graphs 2 and 3.

**Operation of the B&L Spectronic 20 or 340 Spectrophotometer**




1. Turn the spectrophotometer ON using knob A.
   Let the instrument warm up for at least 10 minutes.
   Adjust the meter needle to an Optical Density (O.D.) of 2.0 which is at the far left of the meter. Note that it is not the same as 0% transmission.

2. Select the desired wavelength with the dial D.
   This operates a diffraction grating which diffracts white light to give the various colors (wavelengths) of the spectrum. Wavelengths above 650 millimicrons wide require the use of a special infra-red phototube and filter which are inserted through a trap door in the bottom of the instrument when the current is OFF.

3. Fill a special B&L test tube with water or blank test solution.
   Wipe the tube clean with optical tissue, such as Kimwipe (not Wipettes).
   Insert the tube into the tube adapter (B) with the tube mark facing the mark on the front side of the adapter.
   CLOSE the adapter cover.

4. Adjust the meter needle to ZERO optical density (100% transmittance) using knob E.

5. Repeat the adjustment to O.D. 2.0 and to O.D. 0.0 until the adjustment is stable.

6. The instrument is now ready to read unknowns at this wavelength.
If the B&L tubes are not matched, use the same tube for all measurements.
Rinse, after emptying, with a little of the next solution.

7. If the wavelength is to be changed more than a few millimicrons, reduce the transmittance (increase the O.D.) using knob E before making the change. Afterward reset the instrument to O.D. 2.0 and O.D. 0.0. (See Steps 1 and 4.)

8. Do not turn the instrument OFF until the end of the period or end of the day, or else Steps 1 through 3 must be repeated. However, do not leave tubes in the adapter. The adapter contains a shutter that is closed when the tube is removed and thus the life of the phototube is conserved.

## REPORT SHEET FOR EXERCISE 13

### Enzyme Activity

**Part A. Action of An Inorganic Catalyst**

Tube 1 $H_2O$ plus $MnO_2$ Gas? _____ Did splint flame? _____ What gas? _____

Tube 2 $H_2O$ plus $MnO_2$ Gas? _____ Did splint flame? _____ What gas? _____

Write the chemical reaction for the production of the gas.

**Part B.**

| TUBE | CONTENT | COLOR AT END | DID SPLINT FLAME | RATE OF BUBBLING |
|---|---|---|---|---|
| 1 | Whole liver + $H_2O_2$ + GG* | | | |
| 2 | Liver brei + $H_2O_2$ + GG* | | | |
| 3 | Liver brei + water + GG* | | | |
| 4 | Boiled brei + $H_2O_2$ + GG* | | | |
| 5 | Sand + $H_2O_2$ + GG* | | | |

*Gum guaic

Questions

1. Did whole liver produce gas bubbles as fast as liver brei in tube No. 2?

2. Which reagent, the hydrogen peroxide or the gum guaiac, turned color?

3. What effect did boiling have on the enzyme activity? Why?

4. What effect did grinding have on the enzyme activity? Why?

5. Show what reasoning would go into arriving at a conclusion about the presence of an enzyme which acts on hydrogen peroxide being present in liver.

## REPORT SHEETS FOR EXERCISE 13

**Effect of pH, Concentration and Temperature on the Rate of Enzyme Reactions**

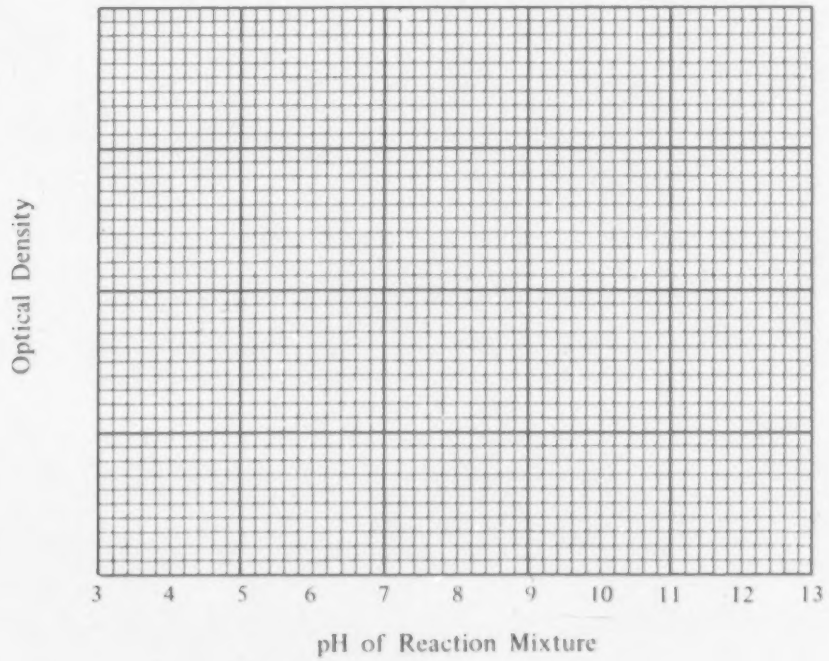
**Experiment 1–Effect of pH on Alkaline and Acid Phosphatase Activity**

Hypothesis:

Results:

| pH | Optical Densities at 405 um. | |
|---|---|---|
| | ACID PHOSPHATASE | ALKALINE PHOSPHATASE |
| 3.0 | | |
| 5.5 | | |
| 7.0 | | |
| 8.5 | | |
| 10.0 | | |
| 12.0 | | |

**GRAPH 1 – Effect of pH on Enzyme Activities**



**Experiment 2–Effect of Temperature and Concentration on Reaction Rate**

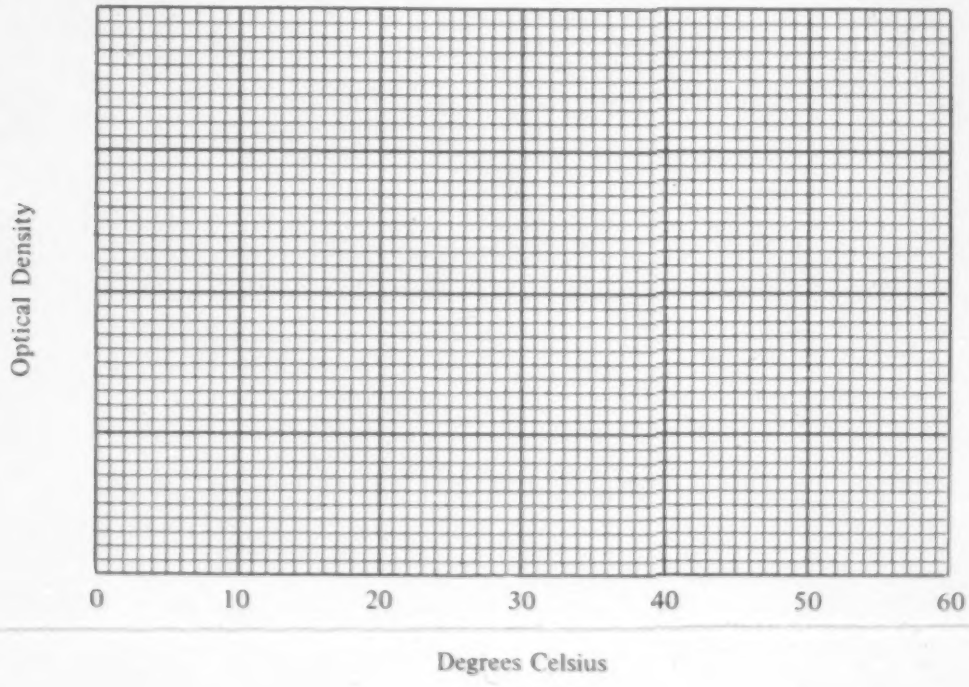
Hypothesis:

Results:

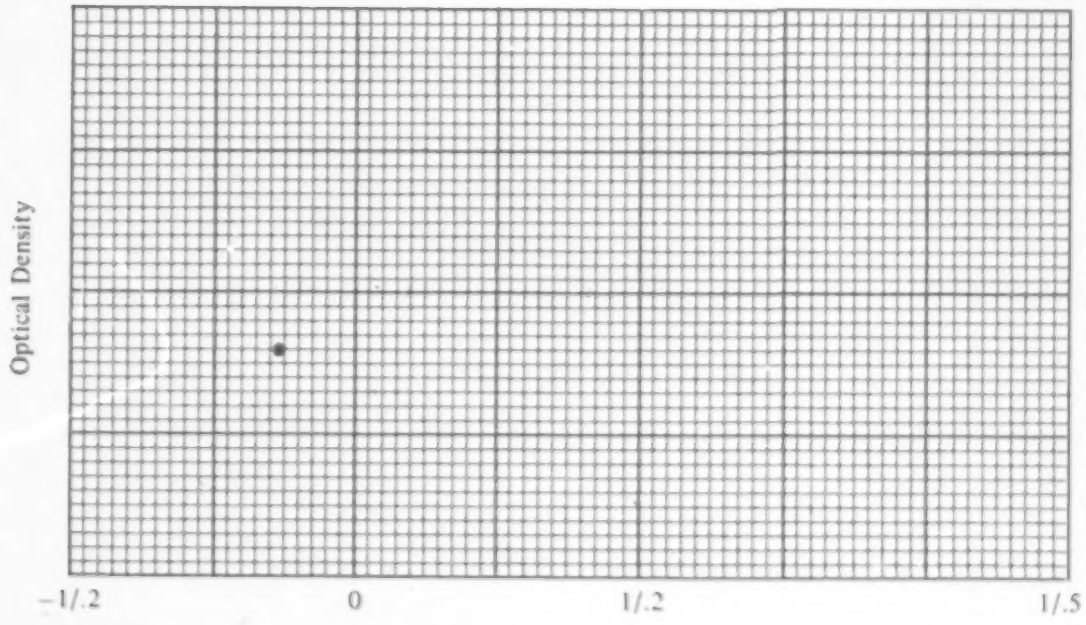
| TEMPERATURE AND CONCENTRATION OF ENZYME | | OPTICAL DENSITIES | | |
|---|---|---|---|---|
| | | TUBE 1 | TUBE 2 | MEAN |
| 0°C. | .5% | | | |
| | .2% | | | |
| 27°C. | .5% | | | |
| | .2% | | | |
| 37°C. | .5% | | | |
| | .2% | | | |
| 57°C. | .5% | | | |
| | .2% | | | |

**Experiment 2** (continued)

**GRAPH 2 – Effect of Temperature and Concentration**




**GRAPH 3 – Lineweaver-Burke Plot**



Questions

1. Based on the results of Experiment 1, what importance can be placed on buffers in the primeval oceans from which the living state arose and what is its significance in modern cells?

2. What would be the value of $Q_{10}$ between 27°C and 37°C, and between 37°C and 57°C? Show your calculations.

Questions

3. Would performing this test for 30 minutes form the basis for a reasonable assay of the amount of enzyme present (within limits)?

4. Why must a blank tube be prepared?

5. Why is it important not to leave tubes in the well of the Spectronic spectrophotometer for very long times?

6. Why is a color produced by this test? Write the reaction.

7. What is the action of enzymes on their substrates? Find a diagram which summarizes the energy relationships of a non-catalyzed and a catalyzed reaction mediated by an enzyme in a text and reproduce it here.

## EXERCISE 14 – FERMENTATION AND AEROBIC RESPIRATION COMPARED

This exercise will probably best be used as a demonstration or as a special project for two or more students

### Part A. Starting Materials and Getting Started

Materials and Equipment

2 2-liter flasks of yeast medium, sterile (prepared in Exercise 7) (Per 8 students)
1 Triple beam balance
3 Aluminum weighing pans
Actively growing yeast culture
2 150-ml. beakers
1 Burette and stand
1 B&L Spectronic 20 or 340 Spectrophotometer and cuvettes
1 box Kimwipes (optical tissue)
Glass tubes
Glass wool
Rubber tubing
1 and 2-hole rubber stoppers
2 250 ml. flasks
250 ml. 0.1N NaOH
Brom-Thymol Blue indicator
3 10-ml. sterile pipette (individually wrapped)
Test tubes and rack
Reagents for glucose determination by the hexokinase method (such as the Gluco-Stat kit from Calbiochem.)

Procedure

1. Each pair of students should weigh the aluminum pans on their triple beam balance as accurately as possible. Record the weights. (Number and initial the pans with some clean, sharp object but not pencil or pen.)

2. With a 10-ml. pipette asceptically transfer 5 ml. of medium to an aluminum pan, and 20 ml. to a beaker. Be careful not to let the pipette touch non-sterile things.

3. Equip the two flasks with sterile tubes and filters as shown below as Form 1 and Form 2.

4. Take a new sterile 10 ml. pipette.
Asceptically transfer 20 ml. of an actively growing yeast culture to each flask. Swirl to mix well.

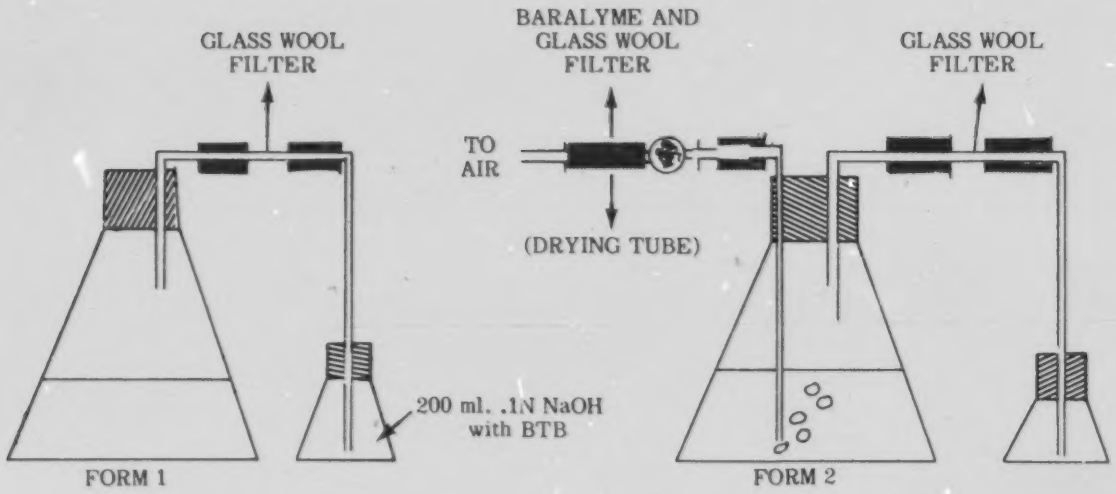
5. Take a new sterile 10 ml. pipette.
Withdraw 10.0 ml. of the flask mixture and divide it between two weighed aluminum weighing pans. Place all three pans of liquid in the drying oven at 100-110°C until dried to a constant weight. Record the dry weight of the yeast (net weight of the yeast samples less twice the weight of the broth sample).

6. Prepare carbon dioxide traps by adding 200 ml. of 0.1N NaOH and enough brom thymol blue to give it a definite color. Start a slow stream of air (about 2 bubbles/sec.) through the Form 2 flask.

7. Transfer 10 ml. of medium from the beaker (Step 2) to another 150 ml. beaker. Titrate it to neutrality with 0.1N NaOH. You may have to use pHydrion paper instead of bromthymol blue indicator because of the amber color of the broth. Record the result.

8. Dilute 1 ml. of the broth (in the beaker from Step 2) to 10.0 ml. with water. This should reduce the concentration from 1% to .1% (100 mg.%). Determine the amount of glucose in the diluted sample using the hexokinase method. (The procedure is provided separately.) Record your results.

9. Hold your results for use next week.




**Part B. Products of Fermentation and Aerobic Metabolism** (Work in pairs)

1 culture each of an aerobic and an anaerobic preparation of yeast 1 week old.
1 Triple beam balance
4 Aluminum weighing pans
1 Fluid trap to vacuum line
(NOTE: Please do NOT connect suction flasks directly to the vacuum line.)
1 Suction flask with Buchner funnels and filter paper.
1 Burette and stand
1 B&L Spectronic 20 or 340 Spectrophotometer with cuvettes
Test tubes and rack
1 250 ml. graduated cylinder and alcohol hydrometer
Reagents for the determination of glucose by the hexokinase method
2 Warburg flasks and manometers Warburg bath
1 Thermometer (°C.)
2N Sulfuric acid
Reagents for the determination of alcohol with alcohol dehydrogenase, such as the STAT-PAK from Calbiochem.
10-ml. pipettes
1-ml. pipettes
Alcohol hydrometer

Procedures

1. Mark the aluminum pans with your initials and number them. Weigh them as accurately as you can. Remove 10 ml. of each culture and divide it between two weighed pans. Place the pans in the drying oven at 100-110°C and dry to a constant weight.

2. Filter the cells out of the culture medium using the Buchner funnel on a suction flask connected to vacuum through a fluid trap. Discard the cells into the container provided.

3. Determine the percent alcohol by volume by filling a 250 ml. graduated cylinder with the filtrates and inserting the alcohol hydrometer. (Rinse and dry it between determinations so as not to contaminate the fluids with each other.) Return the fluids to the appropriate flask.

4. Titrate 10 ml. of each filtrate to neutrality with bromthymol blue or pHydrion paper using 0.1N NaOH. Record your result.

5. Determine the glucose concentration using the hexokinase method.

6. Determine the alcohol concentration in both flasks using the alcohol dehydrogenase method. (The procedure is provided separately.)

7. Determine the amount of carbon dioxide trapped in the .1N NaOH using a Warburg manometer system.

**Determination of Carbon Dioxide (Optional)**

1. Measure the volume of the .1N NaOH in the carbon dioxide traps (flasks) and bring the volume to 200 ml. if there has been any evaporation.

2. Obtain 2 Warburg flasks (one for the fluid in each trap). These are numbered and are calibrated for the correspondingly numbered manometer. The flask constant can be obtained from the bulletin board.

3. Pipette 2 ml. of trapping fluid to the Warburg flask (but not to the center well). Remove the gas port and pipette in 1 ml. of 2 N sulfuric acid to the side arm.

4. Grease the gas port plug with anhydrous lanolin and secure it in place with springs or rubber bands. Grease the manometer tip with lanolin (not too much), put the flask on, and secure immediately with springs or rubber bands. (The flask may stick momentarily without being secured. However, don't leave it or the flask will fall off and break. Open the stopcock at the top of the manometer (blue spot to the right).

5. Place the manometer system on the bath support so that the flask is immersed. Let it equilibrate for about 5 minutes.

6. Close the stopcock and adjust the manometer fluid to 150 mm. with the reservoir knob.

7. Remove the manometer system from the bath and tip in the sulfuric acid. Return to the bath. The carbon dioxide liberated will force the manometer fluid upward in the open arm. To prevent overflow, adjust the closed arm periodically back to 150 mm.

When gas generation is complete, adjust the closed arm to 150 mm. and read the open arm. If the fluid is above the scale (over 300 mm) raise the right hand arm reading so that the left falls to 300. Divide the difference between 150 and the reading on the right arm by 2 and add the quotient to the reading on the right side.

The flask constant is a factor which converts the reading in mm. to microliters of gas at standard conditions of temperature and pressure. Therefore

microliters $CO_2$ = Change in mm x flask constant

Multiply by 100 to get the volume of $CO_2$ trapped in 200 ml. of .1N NaOH.

8. When the determination has been completed, open the stopcock, then remove the manometer from the bath and rinse out the flask.

## REPORT SHEETS FOR EXERCISE 14

### Fermentation and Aerobic Respiration Compared

Dry Weight of Yeast per 10 ml. in Starting Culture

| PAN | GROSS DRY WEIGHT (Gm.) | TARE WEIGHT OF PAN (Gm.) | NET DRY WEIGHT (Gm.) |
|---|---|---|---|
| 2 | | | |
| 3 | | | |
| | | SUM | |
| 1 | | | |

Net Dry Weight of Yeast (Pans 2 + 3 minus 2 x Pan 1) _____

Dry Weight of Yeast per 10 Ml. in Final Anaerobic Culture

| PAN | GROSS DRY WEIGHT (Gm.) | TARE WEIGHT OF PAN (Gm.) | NET DRY WEIGHT (Gm.) |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| | | SUM | |
| 3 | | | |

Net Dry Weight of Yeast (Pans 1 + 2 minus 2 x Pan 3) _____

Gain in Weight (Gm.) _____

Dry Weight of Yeast per 10 ml. in Final Anaerobic Culture

| PAN | GROSS DRY WEIGHT (Gm.) | TARE WEIGHT OF PAN (Gm.) | NET DRY WEIGHT (Gm.) |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| | | SUM | |
| 3 | | | |

Net Dry Weight of Yeast (Pans 1 + 2 minus 2 x Pan 3) _____

Gain in Weight (Gm.) _____

**Titratable Acidity**

| 10 ml. of | pH | Ml. of .1 N NaOH USED | MILLEQUIVALENTS ACID PRESENT |
|---|---|---|---|
| Starting medium | | | |
| Final Anaerobic Filtrate | | | |
| Gain (+) or loss (–) | | | |
| Final Aerobic Filtrate | | | |
| Gain (+) or loss (–) | | | |

**Specific Gravity by Alcohol Hydrometer**

| | % VOLUME | AMOUNT IN WHOLE FILTRATE |
|---|---|---|
| Anaerobic Final Filtrate | | |
| Aerobic Final Filtrate | | |
| Anaerobic Final Filtrate | | |

**Carbon Dioxide Collected in Traps**

| FILTRATE | ml. USED | FLASK NO. | FLASK $k_{CO_2}$ | ULITERS $CO_2$ | TOTAL $CO_2$ TRAPPED | MOLES $CO_2$ VOL/22.4 |
|---|---|---|---|---|---|---|
| From Aerobic Flask | | | | | | |
| From Anaerobic Flask | | | | | | |

**Glucose Content of Mixtures**

| | | O. D. READINGS MINUTES AFTER MIXING | | | | | | Mg % GLUCOSE |
|---|---|---|---|---|---|---|---|---|
| | | $A_0$ | 1 | 2 | 3 | 4 | 5 | |
| Starting Medium | Anaerobic | | | | | | | |
| | Aerobic | | | | | | | |
| Anaerobic Filtrate | | | | | | | | |
| | Method II | | | | | | | |
| Aerobic Filtrate | | | | | | | | |
| | Method II | | | | | | | |

**Alcohol Dehydrogenase Assay for Alcohol in Media and Filtrates**

| | O. D. READINGS MINUTES AFTER MIXING | | | | | | AMOUNT |
|---|---|---|---|---|---|---|---|
| | $A_0$ | 1 | 2 | 3 | 4 | 5 | |
| Anaerobic Filtrate | | | | | | | |
| Aerobic Filtrate | | | | | | | |

## EXERCISE 15 – CHROMOSOME MOVEMENTS DURING CELL DIVISION

(Followed with chromosome models made of wire and pipe cleaners)

*This activity can be assigned for completion before coming to class. Time–30 min.*

Materials

Copper wire (20 to 24 gauge)
Pipe cleaners (may be different colors)
Paper plates (6-in. diameter or larger)

Preparations

1. Cut 8 pieces of pipe cleaner wire about 2 inches long and 8 pieces of 20 to 24 gauge copper wire about 4 inches long. Bend half of these into V shapes and half into J shapes with a small loop at the bend to represent the centromere (where the chromosomal spindle fibers are attached).

2. Cut out the models of the cellular structures provided with this exercise.

**Part A. Procedure for Mitosis**

Mitosis is a form of cell division in which the chromosomes (after replication or duplication) shorten and become more visible in the microscope. They split and are distributed to the resultant cells in sets like those of the parent cell.

While the process is continuous, certain parts of the process are named to help identify the sequences of the process so that scientists may study and discuss them conveniently.

*Interphase*

Place the model of the interphasic nucleus (No. 1) in a paper plate.
Place two copper wire V- and two J-shaped moles of interphasic chromosomes at random in the nucleus. This represents *early interphase*. During interphase the cell will be doing the work it was designed to do so that the chromosomes will largely be concerned with making various kinds of RNA. As readiness for cell division approaches, the chromosomes switch from RNA production to DNA production. This results in the replication (or duplication) of each chromosome, the duplicate lying beside the original.
Place a second copper wire V- and J-shaped chromosome model on the others to form 4 pairs of strands. This represents *late interphase* and indicates readiness for the nucleus to undergo division.

*Prophase*

Place the model of the prophase nucleus (No. 2) in another plate.
Place two pair of J- and two V-shaped pipe cleaner models of chromosomes at random in the nucleus. Towards the end of prophase the nuclear envelope breaks, exposing the nuclear sap to a change in acidity and salinity. This causes the spindle proteins to elongate into the spindle while the prophase chromosomes are still scattered. The time until the chromosomes move to the equator is usually called *prometaphase*.

*Metaphase*

Place the model of the metaphase spindle (No. 3) in a plate.
Place two pair of V- and J-shaped pipe cleaner chromosome models in the spindle so that the small loops representing the centromeres are on the equator.

*Anaphase*

Using the same plate as for metaphase, arrange the chromosome models so that the bends point toward the centrioles but leave the distal tips in touch with the equator. When all have been arranged so that each member of a pair of chromosome models point toward opposite poles, move both sets to the aster on that side.

*Telophase*

Place two models of the telophase nucleus (No. 4) in a plate.

Transfer the chromosomes from the anaphase model to these nuclei. Note that the aster is not fully withdrawn. The beginnings of cytoplasmic division can be seen in cells at this stage. In animal cells a cleavage furrow develops around the equator of the parent cell. In plant cells new cell wall materials are secreted into the equatorial plate so that a division plate is formed there.

*Interphase II.*

Place two interphasic nuclear models (No. 1) in two paper plates.

Supply the nucleus of each with two pairs of copper wires (2 J- and 2 V-shaped models) unpaired at random in the nuclei. The division cycle is thus completed and the cell may return to doing whatever work its new condition is designed to do.

**Part B. First Procedure for Meiosis (Sperm Production)**

Meiosis is a form of cell division in which the chromosomes (after replication) shorten and pair up. After possible breaks and recombinations the tetrads split apart in pairs and are distributed to resultant cells in double, homologous sets. The telophase becomes the prophase of a second division during which chromosomes do not break, but are distributed to the final cells so that each has a single set of homologous chromosomes.

In sperm formation (spermatogenesis) all of the resultant cells are transformed into surviving sperm cells, which is in contrast with what happens in egg production.

*Interphase I.*

Place the model of the interphasic nucleus (No. 1) in a paper plate.

Place two pair of copper wire J- and V-shaped models at random in the nucleus. This represents *early interphase.* As readiness to divide approaches, these strands are duplicated, so place similar models on the original ones and the plate now represents *late interphase.*

*Prophase I.*

Place the model of the prophase nucleus (No. 2) in a plate.

Place a bundle of 4 J- and a bundle of 4 V-shaped chromosome models in the nucleus. These bundles of 4 are called tetrads and result from the matching up of the two pairs of the same form of chromosome (homologous chromosomes) which is a process called synapsis.

*Metaphase I.*

Place the model of the Metaphase spindle in a plate.

Arrange two tetrads of J- and V-shaped chromosomes on the equator.

*Anaphase I.*

Use the same plate as for Metaphase I.

Divide the bundles so that the centromeres of two chromosomes in each bundle are directed toward opposite asters. Move them to the aster end of the spindle.

*Telophase I.*

Place two telophase nucleus models (No. 4) in a plate.
Place a pair of J- and a pair of V-shaped chromosome models at random in each nucleus.

*Prophase II.*

Telophase I is also Prophase II, except that the cytoplasm has to divide.
Place a prophase nucleus model (No. 2) in each of two plates.
Transfer the chromosomes from Telophase I to the nuclei.

*Metaphase II.*

Place models of the metaphase spindle (No. 3) in each of two plates.
Transfer the chromosome models from Prophase II placing their centromeres on the equator.

*Anaphase II.*

Separate each pair of chromosome models so that the centromeres are directed toward opposite asters. Move the chromosomes to the asters.

*Telophase II.*

Place two models of telophase nuclei (No. 4) in each of two plates.
Transfer the chromosome models from Anaphase II to the nuclei.

*Interphase II.*

Transfer each of the Telophase II nuclei to a separate plate.
Replace each pipe cleaner model of a chromosome with a copper wire model to indicate the interphasic condition of these nuclei.

In some animals (Ascaris, Lobster, etc.) the spermatozoan may be amoeboid. However, most males form tailed spermatozoa which are formed from these haploid cells. The centriole generates a long flagellum and the mitochondria become concentrated in the cell so that it is like an ATP-powered torpedo carrying a warhead of one set of chromosomes.

**Part C. Second Procedure for Meiosis (Egg Production)**

In most developing egg cells the spindle size is much reduced in relative size when compared with a developing spermatocyte. However, we will use full-sized spindle models since the process is the same and it will be more convenient working with the larger size.

*Interphase I.*

Place the model of the interphasic nucleus (No. 1) in a paper plate.
Place two pair of copper wire J- and V-shaped models at random in the nucleus. This represents *early interphase*. As readiness to divide approaches, these strands are duplicated, so place similar models on the original ones and the plate now represents *late interphase*.

*Prophase I.*

Place the model of the prophase nucleus (No. 2) in a plate.
Place a bundle of 4 J- and a bundle of 4 V-shaped chromosome models in the nucleus. These bundles of 4 are called tetrads and result from synapsis of pairs of homologous chromosomes.

*Metaphase I.*

Place the model of the metaphase spindle in a plate.
Arrange two tetrads of J- and V-shaped chromosomes on the equator.

*Anaphase I.*

Use the same plate as for Metaphase I.
Divide the bundles so that the centromeres of two chromosomes in each bundle are directed towards opposite asters. Move them to the aster end of the spindle.

*Telophase I.*

Place one telophase nucleus model (No. 4) in a plate and place a model of the polar body cell (No. 5) at the opposite pole outside the plate.
Place one set of chromosome models from Anaphase I at random in the nucleus in the plate and the other set in the nucleus of the polar body cell.

*Prophase II.*

Telophase I for the cell in the plate is also Prophase II.

*Metaphase II.*

Place a spindle model (No. 3) in a plate.
Transfer the chromosomes from the plate used for Telophase I to the Metaphase model, placing the centromere loops on the equator.

*Anaphase II.*

Separate the pair of chromosomes in both the J- and V-shaped groups. These same changes are taking place in the polar body. Move the chromosome models to the aster end of the spindle.

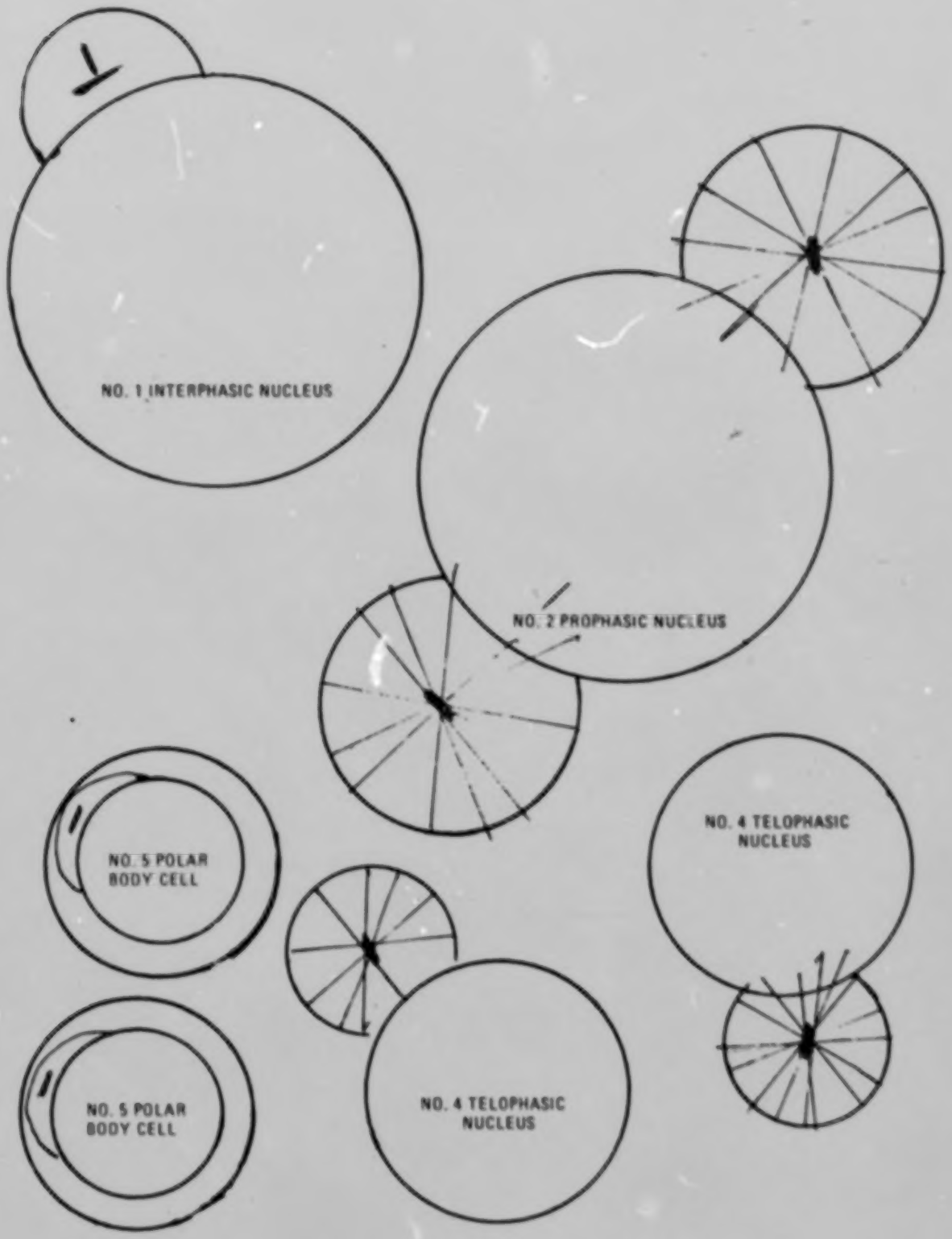
*Telophase II.*

Place a telophase nucleus model (No. 4) in a plate.
Transfer one set of chromosomes from Anaphase II to this nucleus. Transfer the other set to a polar body cell model (No. 5) beside the plate. Place two more polar body cell models (No. 5) beside the polar body cell model for Telophase I. Divide the chromosome pairs and put one set in one of the polar body models and the other set in the other.

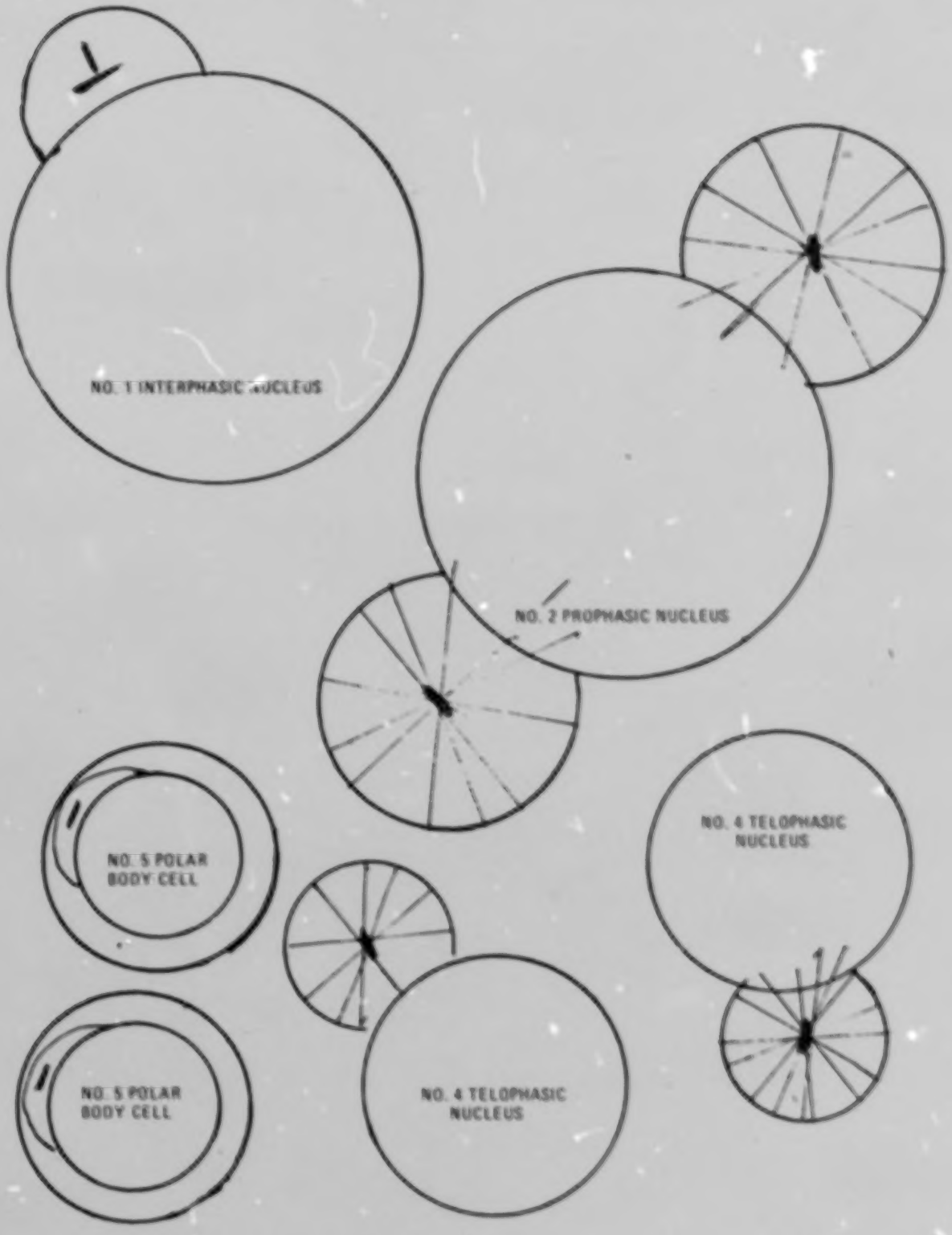
*Interphase II.*

The plate model in Telophase II is a model of an ovum. You will have noted that as in sperm formation there are now four cells, but three of them are somewhat reduced in size and in fact they are not functional as eggs, because they lack cytoplasm to store food for developing embryos. The model now represents the egg pronucleus. The chromosomes will go immediately to prophase as will the chromosomes of the sperm pronucleus. The sperm pronucleus has the centrioles for the zygotic spindle. The pronuclei will not "fuse," but rather the chromosomes of the female pronucleus will enter the spindle of the male pronucleus, and the yoking (zygote formation) of the two sets of chromosomes is completed.

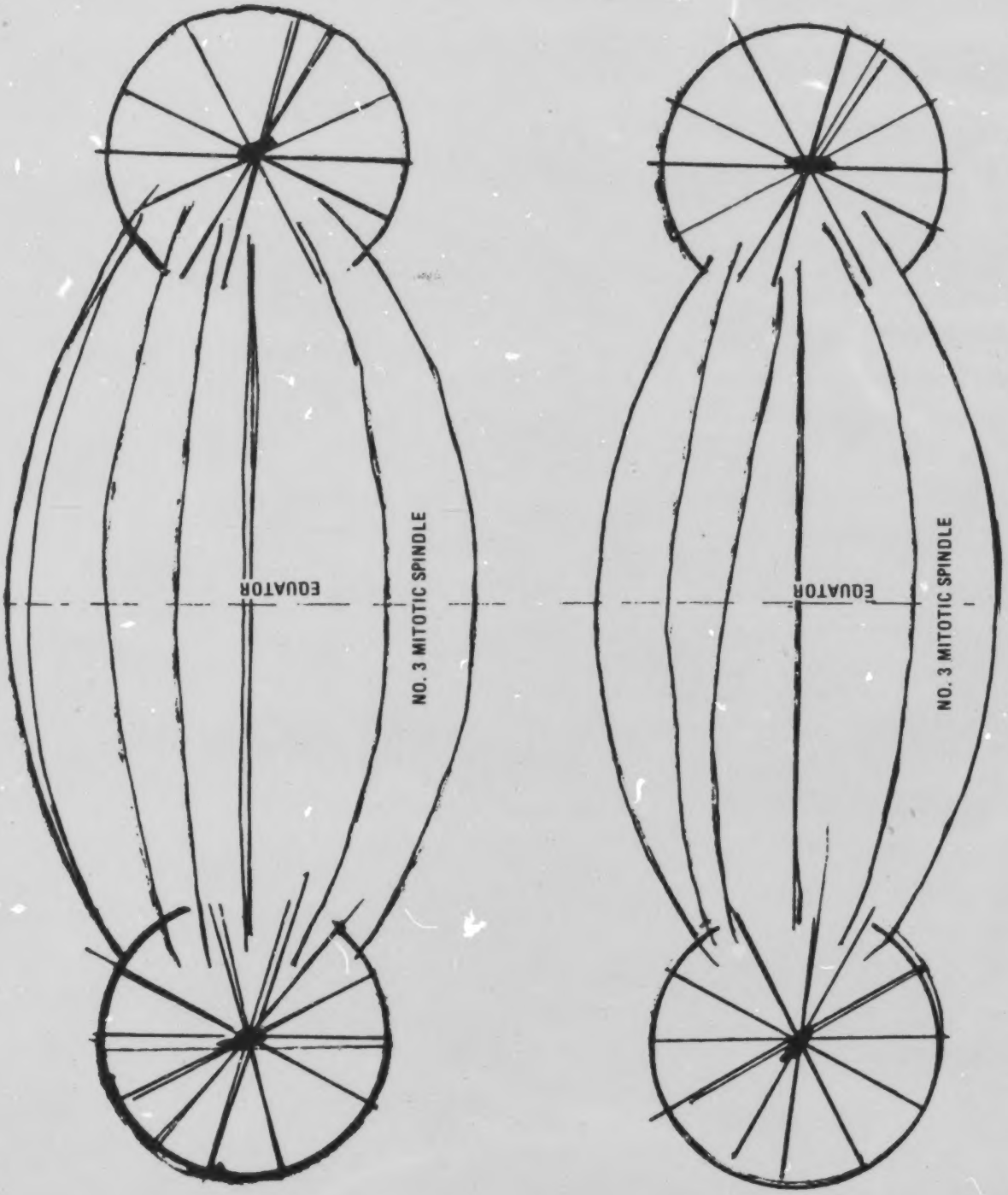
Nuclear, Astral and Spindle Models to be Cut Out and Placed in Paper Plates


NO. 1 INTERPHASIC NUCLEUS
NO. 2 PROPHASIC NUCLEUS
NO. 5 POLAR BODY CELL
NO. 4 TELOPHASIC NUCLEUS
NO. 5 POLAR BODY CELL
NO. 4 TELOPHASIC NUCLEUS

Nuclear, Astral and Spindle Models to be Cut Out and Placed in Paper Plates



**Nuclear, Astral and Spindle Models to be Cut Out and Placed in Paper Plates**



# EXERCISE 16 – CELL DIVISION

Material and Equipment

Microscope with lamp
Razor blade
Microscope slides and coverslips
Forceps, dropper and dissection needle
Chart of animal and plant mitosis
Aceto-orcein light green stain
Prepared slides of whitefish blastula
Prepared slides of maturation in Ascaris eggs
Chloroform
Absolute alcohol
Glacial acetic acid
Allen's Fluid
Acid alcohol

## Part A. Demonstration of Mitotic Figures in Onion Root Tip[1]

These slides can be prepared in as little as three minutes.

Step 1. Cut off 1/8-inch of the root tip with a pair of scissors. Pick up the tip with the forceps. (It is best to select small onions for growing the roots and to allow the roots to grow over 3/4 of an inch in length before cutting the tip.) These roots must be growing vigorously.

Step 2. Place the tip in Allen's Fluid for 35-40 seconds; then blot.

Step 3. Then place the tip in Acid-Alcohol for 35-40 seconds; blot.

Step 4. From the Acid-Alcohol place the tip on a slide and then add several drops of Aceto-Orcein-Light Green stain on the tip.

Step 5. With a glass coverslip apply pressure on the tip until it becomes smeared thinly. Then slowly lift the coverglass off the slide, add more stain, and allow the stain to get to the smeared portions. Press on the smear again. This lifting and pressing should be done three times.

Step 6. Leave the smeared root tip in the stain approximately 3-4 minutes. Within ten minutes (of hunting) student should find good mitotic figures.

**Allen's Fluid** (Fixative and Mordant)

Saturated solution of picric acid in water . . . . . 75 cc.
Formalin, U.S.P. . . . . 15 cc.
Acetic Acid, Glacial . . . . . 10 cc.
Urea . . . . . 1 gm.

**Acid Alcohol** (Softener)

Concentrated Hydrochloric Acid . . . . . 100 cc.
Iso-propyl Alcohol, 99% . . . . . 200 cc.

**Aceto-Orcein-Light Green Stain**

Solution 1 . . . . . 1 part  Solution 2 . . . . . 1.5 parts

[1] Courtesy Reid Jackson, Graduate School of Education, Harvard University

**Solution 1**: 0.125 grams of Light Green S.P. dissolved in 100 cc. of a 50% Aqueous solution of Acetic Acid, and filtered.

**Solution 2**: 0.5 grams of Orcein dissolved in 100 cc. of 50% Aqueous solution of Acetic Acid, and filtered.

Mix just before using.

**Part B. Mitosis**

The whole study of mitosis is simplified and made more significant if certain basic ideas are kept in mind throughout the observations. Every cell must descend from a pre-existing cell and daughter cells resemble the parent cell. It follows then, that cell division is equational in character, the two daughter cells being equal to each other and to the parent from which they derived. Since we know that the shape and the size and the activities of a cell are controlled by factors in the nucleus, we must examine critically the fate of the nucleus as it undergoes division to form two daughter cells.

It is important to bear in mind that the phases of mitosis you observe are like the single shots in a motion picture film. Understand the single dynamic of the whole process as it goes on in living growing cells. Observe the reversing character of mitosis and that the changes from prophase to metaphase are roughly like those from metaphase to telophase.

To observe mitosis in living material it is necessary to secure tissue where growth is rapid and cells are actively dividing. Mitosis is a universal phenomenon and might be observed in the lining layers of your throat or about a healing wound. It is generally preferable to use vegetable tissue.

Nuclear material and chromosomes have a greater affinity for the stain than other cell parts. Observe the loosely compacted cells of the root cap. Can you think of a use for these in the economy of root growth? Beneath the root cap at the very tip of the root proper will be observed a mass of small densely cytoplasmic cells. Look for actively dividing cells with chromosomes in this region. Back of the tip will be seen a zone of larger and longer cells appearing more or less empty. This is the zone of cell enlargement including some incipient conducting cells with springlike coils of cell wall thickenings.

Supplement your examination of the fresh root tip with prepared slides of onion root tips showing mitosis. Draw a series of figures showing what you see in typical onion root cells undergoing mitosis. Arrange your drawings in a series to show the true sequence–early prophase to late telophase. Resting nuclei are characterized by a uniformly granular appearance. During prophase this granular material becomes organized into long slender much convoluted threads which become progressively shorter and thicker. Prophase nuclei also show a small dark staining spherical body–the nucleolus. This structure is of uncertain function but disappears at the end of prophase.

The prophase figure is succeeded by metaphase. At this stage the nuclear membrane has completely disappeared, and a spindle figure has formed. The spindle is actually formed of more densely gelated protoplasmic strands. Keep in mind its three dimensional aspects. Note that the chromosomes are now obviously paired structures formed by the lengthwise splitting of the parent chromosomes. (The actual splitting occurred in early prophase, but is difficult to see in prophase figures.) In onion there are fourteen long chromosomes. You will note that they extend well up and down the spindle figure but that a plane passed through the equator of the spindle would touch each chromosome. Each chromosome has a point of attachment to the spindle figure which in the metaphase figure always lies in this equatorial region. Metaphase is a *one-condition figure*. We do not speak of "early" and "late" metaphase.

During anaphase the chromosome halves move apart, so that each leaves its twin of the metaphase and moves toward opposite poles of the spindle figure. The anaphase is dynamic and may be considered to last only while the chromosomes have a V-shape with the point directed toward the pole where a daughter nucleus will be reconstituted. This chromosomal movement is not fully understood, but it is thought that they are drawn along by tractile fibers which some workers believe to be *pseudopodium-like extrusions of the chromosome itself.*

Telophase begins when movement of the chromosomes stops. During this phase the two chromosome groups reorganize as new nuclei with the chromosomes losing their gelatinous matrix and becoming successively threadlike and granular again as in the prophase. Other telophasic changes in plant cells include the reappearance of nucleoli and nuclear membrane together with formation of a "cell plate" in the equatorial region. By the coalescence of droplets this cell plate becomes the cell wall of late telophase and cell division is complete. Note that the cell plate and cell wall divide the cytoplasm in two parts. There is no elaborate mechanism for precise equational division here.

Follow your observations of plant mitosis with a study of prepared slides of whitefish blastulae as examples of mitosis in typical animal cells. The blastula is an early embryonic stage following fertilization of the fish egg. Make a record of differences between this and division in a plant cell. See aster, central bodies and try to find a centriole. Do you find any asters which have appeared before the breakdown of the nuclear membrane? Note the pinching in two of the parent cell as the two daughter cells are formed in late telophase. What is the mechanism of such a construction of protoplasm? This question is still unanswered by modern researchers.

In using the prepared slides of both the onion root and the whitefish blastula remember that the effect of sectioning is to show only parts of cells in most cases. You could not understand the shape of a whole bologna by examining a single slice. Examine many cells and in your own mind reconstruct the situation in an intact undamaged cell.

**References**

Mazia, D. 1953. Cell Division. *Scientific American.* August, 53–63.

Swanson, Carl P. 1960. *The Cell.* 2nd ed. Englewood Cliffs, New Jersey. Prentice-Hall, Inc. pp. 62–77.

**Part C. Meiosis**

Study a slide of maturing eggs of *Ascaris megalocephala,* the round worm of horses. This is convenient material because there are only two chromosomes to follow. These eggs, however, like many invertebrate animal eggs, become *ripe* for sperm entrance before the meitoic divisions begin in the egg. The sperm nucleus, on the other hand, waits in the egg cytoplasm for the meitoic division to be complete before the male pronucleus fuses with the female pronucleus. Find these stages:

*Germinal vesicle stage.* This is the egg before sperm entrance. It has a large nucleus (germinal vesicle) which is not usually in the center of the cell. The cell is surrounded by a jelly coat and there is a perivitelline space between the jelly coat and the egg cell itself.

*Prophase.* Two chromosomes will be seen in the female nucleus. The sperm nucleus is a deep-staining body off to one side.

*Metaphase I.* Two tetrads of chromosomes can be seen in the spindle at the edge of the egg cell.

*Metaphase II.* The first polar body cell has been formed and may be mashed against the jelly coat. Two diads (pairs) of chromosomes will be seen in the spindle near the edge of the egg cell.

*Pronuclear Stage.* The second polar body cell will now be pinched off the edge of the egg cell and the egg pronucleus is approaching the vesicular male pronucleus and its asters. The female chromosomes will enter the spindle formed by the male pronucleus and the first mitotic division of the zygote will ensue.

## REPORT SHEETS FOR EXERCISE 16

**Drawings of Onion Root Tip Cells in Various Stages of Mitosis**

Stain Used: Drawn from a magnification of _____X

**Drawings of Cells in the Whitefish Blastula in Various Stages of Mitosis**

Stain Used: Drawn from a magnification of _____ X

**Drawings of Ascaris Eggs in Different Stages of Meiosis**

Drawn from a magnification of _____ X

**Cell Division**

1. Why are cells stained to study mitosis if staining them results in their death?
2. If a cell has four pairs of chromosomes, how many pairs will each daughter cell have following mitosis?
3. Briefly characterize the mitotic phases.
4. What do you think the role of spindle fibers is?
5. How are plant mitosis and animal mitosis similar?
6. What major structural differences are there between mitosis in onion cells and whitefish blastula cells?
7. What is synapsis?
8. What are polar body cells?
9. How do the final cells in male mitosis differ in viability with the final cells in female meiosis in animals?

Use additional sheets of this size to complete your answers, if necessary.

## EXERCISE 17 – THE PHYLA OF THE ANIMAL AND PLANT KINGDOMS

There will be placed on demonstration a representative specimen for each of the major phyla in the plant and animal kingdoms. Phylum means "tribe," and therefore is made up of several Classes, Orders, Families, Genera and Species. You will be asked in this exercise to identify the main structural characteristics that distinguish members of one phylum from another. This is almost like asking a "man from Mars" to look at you and characterize Earthmen as well as Americans as a nation. On the other hand it would be easier for him to take you back to Mars with your characteristics than to transport the whole city of Atlanta, Georgia back to the red planet for the same purpose.

A basic chart has been provided for your guidance as the answer sheet for this exercise.

## REPORT SHEETS FOR EXERCISE 17

| PHYLUM | ANIMAL KINGDOM | |
|---|---|---|
| | NAME OF ANIMAL OBSERVED | STRUCTURAL CHARACTERISTICS |
| Porifera | | |
| Coelenterata (Cnidaria) | | |
| Platyhelminthes | | |
| Nematoda | | |
| Bryozoa | | |
| Brachiopoda | | |
| Mollusca | | |
| Annelida | | |
| Arthropoda | | |
| Echinodermata | | |
| Chordata | | |
| SUBPHYLUM VERTEBRATA | | |
| Class Elasbranchii | | |
| Class Pisces | | |
| Class Amphibia | | |
| Class Reptilia | | |
| Class Aves | | |
| Class Mammalia | | |

| PHYLUM OR CLASS | PLANT KINGDOM | |
|---|---|---|
| | NAME OF ORGANISM OBSERVED | STRUCTURAL CHARACTERISTICS |
| PHYLUM THALLOPHYTA | | |
| Class Schizomycetes | | |
| Class Myxomycetes | | |
| Class Algae | | |
| Class Fungi | | |
| Class Ascomycetes | | |
| Class Fungi Imperfecti | | |
| PHYLUM TRACHEOPHYTA | | |
| Class Bryophyta | | |
| Class Pteropsida | | |
| Class Gymnospermae | | |
| Class Angiospermae | | |

## EXERCISE 18 – COMPARATIVE ANATOMY OF SOME SKELETAL FEATURES

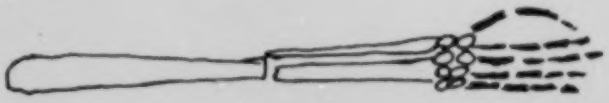
Materials

Skeletons of fish, frog, lizzard or turtle, bird, bat, cat, horse, cow or pig, monkey or man. Clipboard with drawing paper. Pencils

Procedure

Make simple line diagrams (not drawings) of the bones in the arm, wrist and hand of the above animals and under each tell what the appendage is used for; that is, its capabilities.

Example:



Man

The joint moves easily at the shoulder, bends together at the elbow and has universal action at the wrists. The jointed fingers can close into a fist with an opposable thumb for picking up small objects. The capabilities of this organ are throwing, swimming, hammering, caressing, squeezing, waving, receiving, paddling, and many others.

## EXERCISE 19 – DISSECTION OF THE FETAL PIG

Materials and Equipment

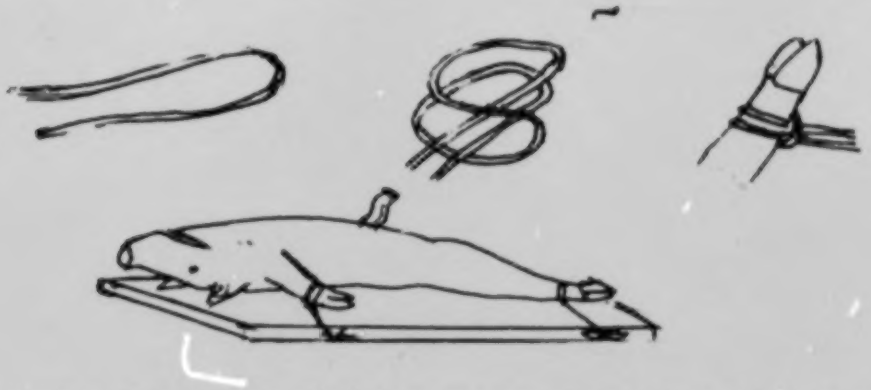
Dissecting board or pan
String
Dissection instruments: Blunt-sharp scissors, blunt probe, forceps, scalpel or single-edged razor blade
Fetal pigs with vessels injected with latex
Hydrous lanolin
Plastic bag and name tag

Procedure

1. Rub your hands with hydrous lanolin to protect them from the preservative.

2. Wash the fetal pig assigned to you with running tap water to remove excess preservative.

3. Secure the animal to the dissecting board by placing it on its back and putting a loop of string around each leg, then tie these to the board, viz:



4. Use a scalpel or single-edged razor blade to make a crosswise cut, about an inch long and an inch anterior (toward the head) to the stump of the umbilical cord. Insert the blunt blade of the scissors into the incision and cut laterally to both sides of the abdominal cavity.

5. Extend the cuts anteriad (toward the head) through the rib cage.
   Extend the cuts posteriad to the bottom of the body cavity.
   Reflect the anterior flap over the head and the posterior one over the tail.

6. Rinse the preservative with running tap water. Disturb the organs as little as possible.

7. Identify these structures and parts:

The Abdominal Cavity

This is lined with a thin, glistening membrane, the *peritoneum* which covers the walls of the cavity and the surfaces of the *visceral* organs. Where these are in the cavity, the connections are called *mesenteries*. The mesenteries supply the intestine, for example, with *blood vessels* and *nerves*.

The Chest Cavities

The chest cavity is separated from the abdominal one by the *diaphragm*, a saucer-shaped sheet of muscle. The pinkish lobes of the *lungs* are easily identified. Air reaches them through respiratory tubes, the *bronchi* and *trachea*. Surrounding the lungs is a thin membranous sac, the *pleura* and the contained pleural cavity. The membranes over the *heart* are called the *pericardium* and enclose the *pericardial cavity*.

The Viscera of the Abdominal Cavity

The *liver* is relatively large and brown in color. It lies next to the *diaphragm*. Next to it is the stomach. The stomach empties into the convoluted *small intestine*, which occupies most of the right side of the body cavity. The *large intestine* empties into the rectum and ends at the anus. The *pancreas* is found in the angle of the stomach and the small intestine. The *spleen*, part of the lymphatic system, is an elongated, reddish-brown body next to the *stomach*. Without disrupting the mesenteries, move the intestines aside to see the *kidneys*, two bean-shaped, brown organs embedded in the posterior wall of the body cavity. Embedded in the fatty tissue at the top of the kidneys are the *adrenal* (or superrenal) *glands*. Posterior to the kidneys, find the whitish *urinary bladder*. At the posterior end it narrows into a duct, the *allantoic duct*, which extends into the fetal end of the umbilical cord.

The Fetal Circulation

In the cut end of the *umbilical cord* identify the collapsed *umbilical vein* and the open umbilical arteries. The *allantois* may also be seen, depending on the age of the fetus and the distance from the fetus the cord is cut. Inside of the lower skin flap, recognize the umbilical vein leading forward to the liver. The umbilical vein may have been cut. Posteriorly, the umbilical arteries, one from each hind leg, enter the stalk of the cord.

Organ Systems

Organs seldom function independently. Therefore, those organs that function together are grouped into organ systems. Each organ performs a special set of functions for the body but some organs cannot do well without its system-mates. For example, a large cut in a blood vessel (an organ) may result in enough blood loss so that the heart doesn't have enough to pump. Since it cannot propel blood to the lungs and back to the rest of the body, cells all over the body begin to die. Below is listed the organs in a few systems.

The organ systems of the human body are generally identified as the muscular, nervous, sensory, circulatory, lymphatic, respiratory, digestive, excretory, endocrine, skeletal, reproductive, and integumentary systems. When the organ systems are morphologically integrated and physiologically coordinated, that is an organism.

Below are lists of the organs in a few systems.

*Integumentary System*

Skin
Nails
Teeth
Hair

*Circulatory System*

Fetus:
Umbilical arteries and vein to the placenta
Heart
Aorta and other arteries
Superior and Inferior vena cava and other veins
Capillaries

*Respiratory System*

External nares
Nasal cavity
Pharynx
Larynx
Trachea
Bronchi and Bronchioles
leading to alveoli in the
Lobes of the lung

*Digestive System*

Mouth (buccal cavity)
Pharynx
Esophagus
Stomach
Small intestine with its glands,
the liver and pancreas
Large intestine with
Cecum and Vermiform Appendix
near its beginning
Rectum
Anus

*Excretory System*

Lungs (part of the respiratory system)
Sweat glands (part of integument)
Urinary system
Large intestine (digestive system)

*Urinary System*

Kidneys
Ureters
Urinary bladder
Urethra (to the outside)

*Reproductive System (Female)*

Ovaries
Fallopian tubes (oviducts)
Uterus (womb)
Vagina
Vulva (external folds)

*Reproductive System (Male)*

Testes (in a scrotum in some mammals)
Epididymis
Ductus deferens leading past
seminal vesicles to the
Prostate gland
Urethra through the penis
(external genital organ)

**Reference**: Humphrey, D. G., H. van Dyke and D. L. Willis. 1969. *Life in the Laboratory* (Shorter Edition) New York. Harcourt, Brace & World. 209 pages. Pages 101–132.

## REPORT SHEET FOR EXERCISE 19

### Dissection of the Fetal Pig[1]

1. What structures are seen in the fetal pig that are not present in the adult? Relate these structures to prenatal function.

2. After identifying external anatomical features of the pig, compare these features with the human body.

3. Pick out the structures you have examined that belong to a particular organ system. Are they all together? How are these structures related? What functional significance is there in their arrangement, structure, and location?

4. Trace various pathways in the specimen; e.g., air, food, blood, urine, eggs or sperms.

[1]Questions courtesy Prof. Muriel E. Taylor, Talladega College.

# EXERCISE 20 – SEXUAL REPRODUCTION AND VEGETATIVE REPRODUCTION (WITHOUT RESPECT TO SEX)

This exercise contains a number of parts. You will probably be asked to do one of the life cycles, but with the permission of the teacher you may do more than one.

Materials and Equipment

Microscope and lamp
Microscope slides and coverslips
Living cultures or preserved specimens for the cycle(s) you will study
Dissecting kit

**Introduction: Life Cycles**

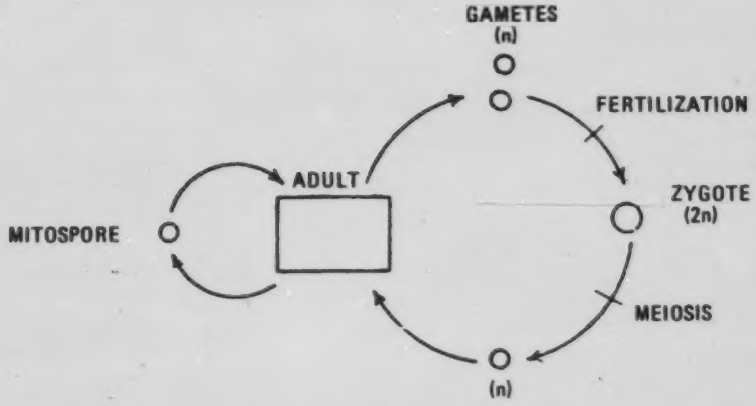
All living organisms have come from living predecessors which in turn have come from a long line of predecessors reaching all the way back to the first living cell or cells. Reproduction has been the overall term used for the process whereby the living state is passed on to new generations of cells and organisms before the parent body dies. Sexual types of reproduction involve the combining of chromosomal factors from two (or more) cells to form a zygote (yoke). However, all cells have sex, and frequently cells divide without combining with other cells. Therefore, mitosis in single-celled organisms is reproduction of the individual. Mitosis is also the basis of budding and many other forms of vegetative reproduction. This phenomenon has been called "asexual" for years, despite the fact that all cells have sex. We, therefore, prefer "vegetative reproduction" or "reproduction without respect to sex" for those forms that do not involve the combination of cells (sexual reproduction). Most organisms carry a double set of chromosomes (which, generally, do not have identical materials in them). There are stages in the lives of most organisms when some or *all* cells have only one set of chromosomes (in the germ cells). Since the number is constant for each species, under normal conditions, we denote the single set of chromosomes as one genome and represent it by the letter n (for basic number), and then call the double (or diploid) set 2n, a triple set (triploid) 3n, etc.

Despite this diversity only three basic patterns emerge into which we can fit the reproductive cycles of almost all living organisms.

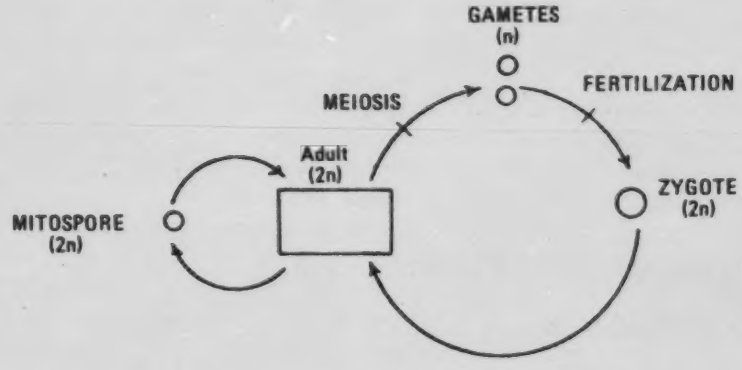
HAPLONTIC patterns are the most primitive and are the most common among the simple one-celled organisms making up the Monera and Protists. In this pattern meiosis occurs after "fertilization," "syngamy," "conjugation" or whatever term we apply to the exchange of genetic material. The haploid organisms may reproduce by any of the three basic patterns.

DIPLONTIC patterns–gametes are produced and sexual reproduction is usual. However, these organisms may also use the other two patterns on occasion. When sexual reproduction modes are used, then meiosis precedes fertilization so that the haploid condition only lasts as long as the gametes are not united.

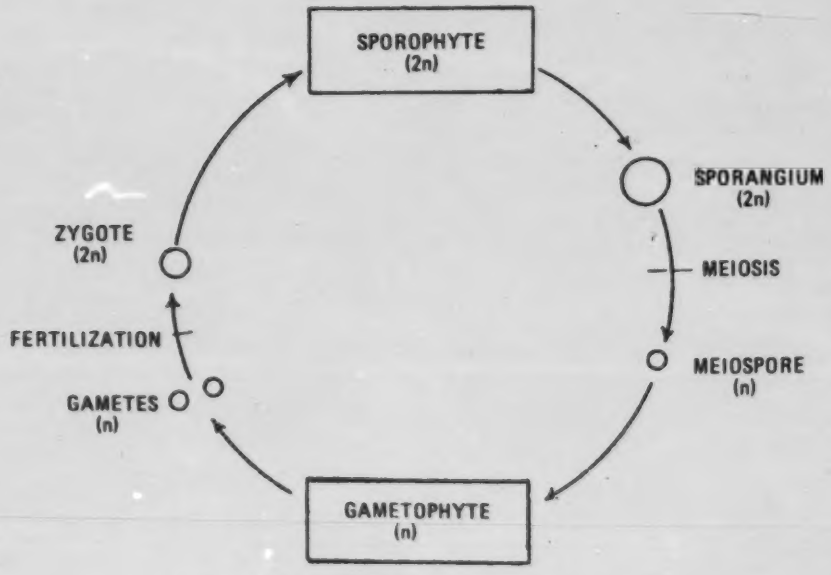
DIPLOHAPLONTIC patterns alternate generation of haploid and diploid individuals. The diploid (2n) individual produces haploid cells which grow into haploid organisms. These haploid organisms then form reproductive cells (gamets) which fuse at fertilization to form diploid form individuals again.




The Haplontic Pattern of Life Cycling




A Diplontic Pattern of Life Cycling




A Diplohaplontic Pattern of Life Cycling

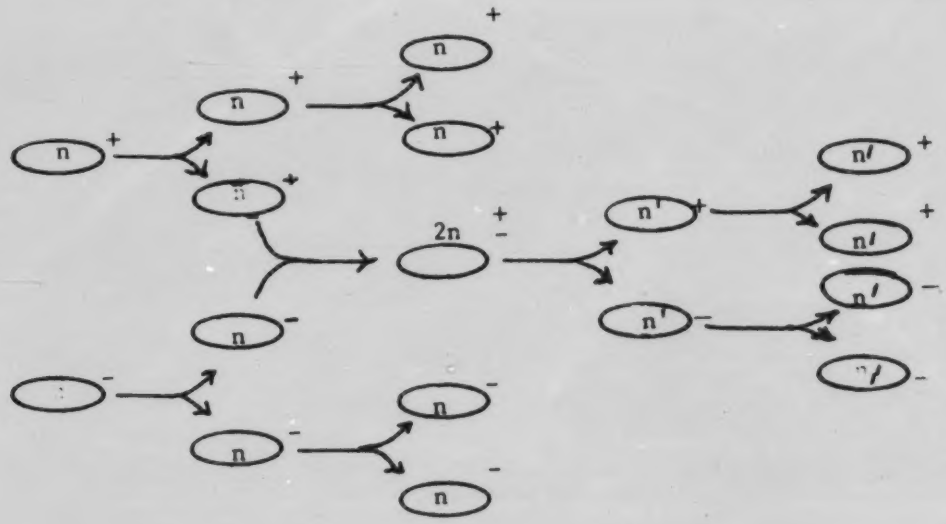
**Part A. Reproduction in Monera**

1. Bacteria

When studied by the light microscope no chromosomes have been seen in bacteria. However, nuclear material has been demonstrated by staining and viewing in the light microscope and the nuclear material is easily identified in the electron microscope. Many heredity characteristics of bacteria have been mapped genetically so that the presence of gene linkages is certain and in fact loop type chromosomes have been photographed in the process of dividing (viewed with the electron microscope).

Because no mitotic spindle has been seen, one may argue that this is not mitosis but *binary fission* of the nuclear material. However, the process of separating the genic material into two equivalent parts, each going to one of the resultant cells is exactly what happens in mitosis and that is what the process should be called.

Bacteria of opposite mating types (male and female or + and –) may mate (called conjugation, an old word meaning marry). This is accomplished by means of a fine cytoplasmic bridge across which the "male" injects its chromosome(s). The zygote thus formed is diploid, but soon undergoes a meiosis resulting in new haploid cells again.




Reproduction in Bacteria. The conjugation of opposition mating types results in a 2n zygote which undergoes meiosis to yield resultant cells of type n′. These (n′) cells show genetic variation because of crossing-over between the chromosome strands.

2. Blue-Green Algae

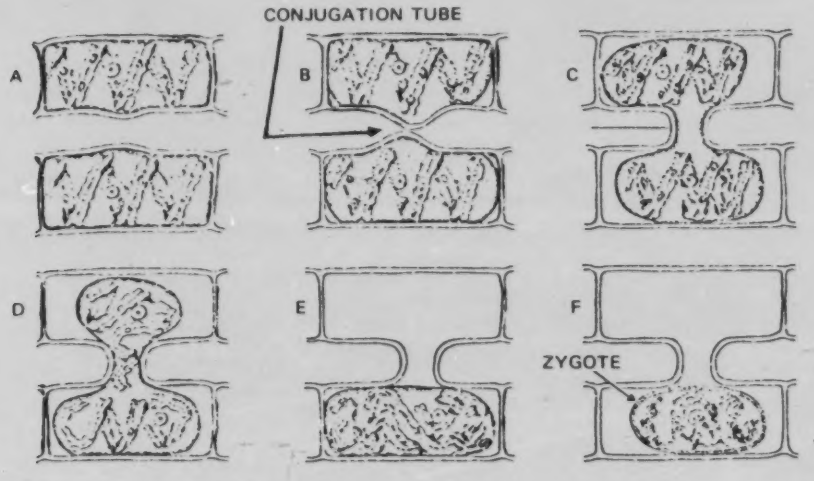
The nuclear material is not in a single mass of material but separated into a number of granules. The protoplasm within the cell may be divided into many spore cells, or spores may be formed in a linear series at one end of a cell in sporulative types of reproduction. This is referred to as fragmentation, bu' that term is based on now outmoded means of observation. As in bacteria, the algal filament can also reproduce mitotically.

**Part B. Reproduction in Protista**

1. Algae

*Spirogyra* is a filamentous green alga which microscopic examination shows to have a large spiral chloroplast. This alga is often part of the green scum seen at the edges of stagnant pools and ponds. The cells divide mitotically during the spring and summer but as the shorter, cooler days of fall approach they begin to conjugate and form zygospores.

Examine both living and preserved specimens. Perhaps the instructor may have a series of prepared slides with all of the stages on them. Identify all of these stages:




Life Cycle of Spirogyra

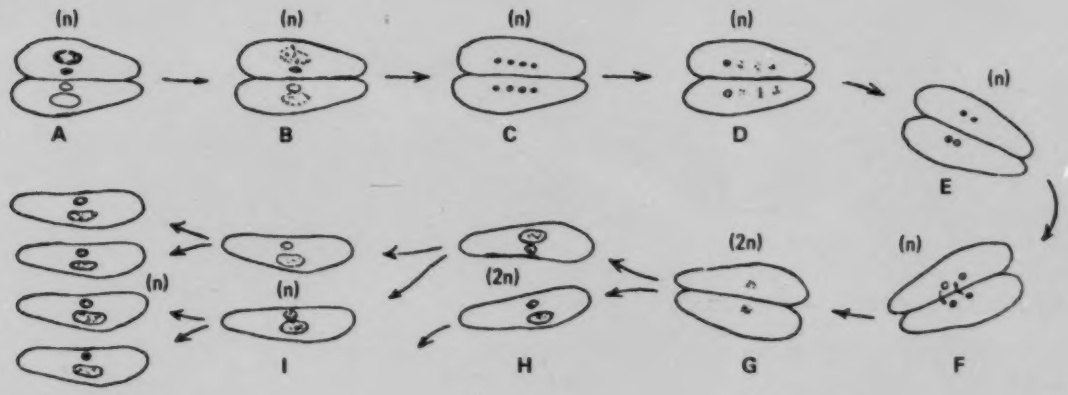
2. Protozoa

Vegetatives reproduction by mitotic (spindle-less) cell division is the principle process for cell reproduction among the Protozoa. However, some forms, especially the ciliates and flagellates, may undergo conjugation resulting in genetic transfers resulting in organisms which may be better adapted to their environment.

Examine a slide of Paramecium showing cells in conjugation and transverse fission.

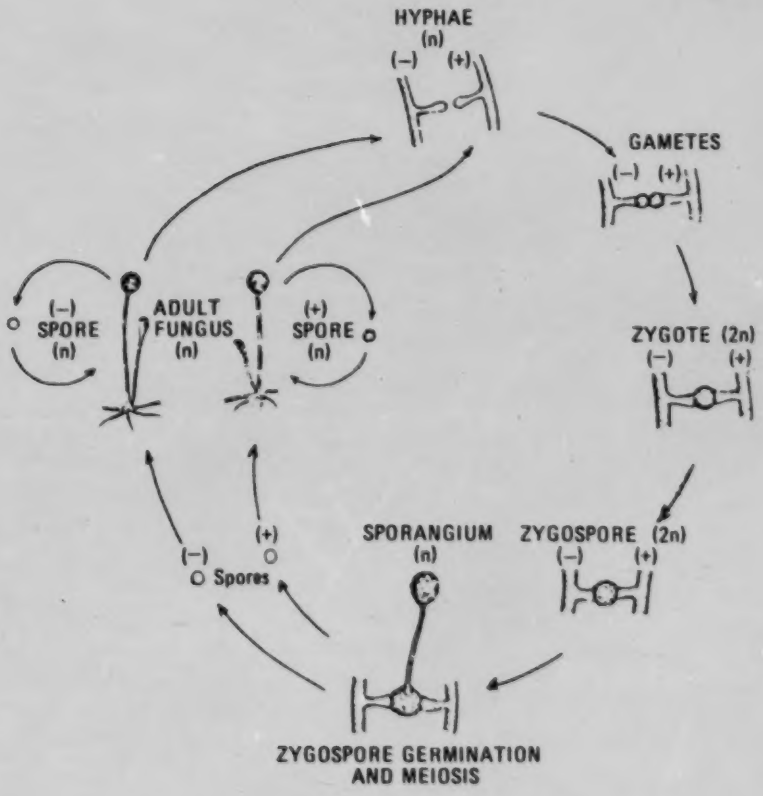
3. Fungi

Reproduction by sporulation and gamete formation is characteristic for the fungi. Study material from a culture or a prepared slide of *Rhizopus nigricans*, a member of the Class Phycomycetes. You will notice that the reproductive structures are formed only when hyphae of the opposite mating types are in close associations.




A. Union to Form Mating Pair
B. Degeneration of Macronucleus
C. Micronucleus Divides Twice
D. Three Micronuclei Degenerate
E. Micronucleus Divides to Form Gamete Nuclei
F. Gamete Nuclei (n) are Exchanged
G. Zygote Nucleus (2n) Formed by Gamete Nuclei Fusion
H. Macronucleus Formed from Micronucleus After Individuals Separate
I. Each Individual Divides by Meiosis to Form Four Individuals (n)

Conjugation and Meiosis in the Ciliate, Paramecium




Life Cycle of the Mold *Rhizopus Nigricans*

4. Yeasts and Other Ascomycetes

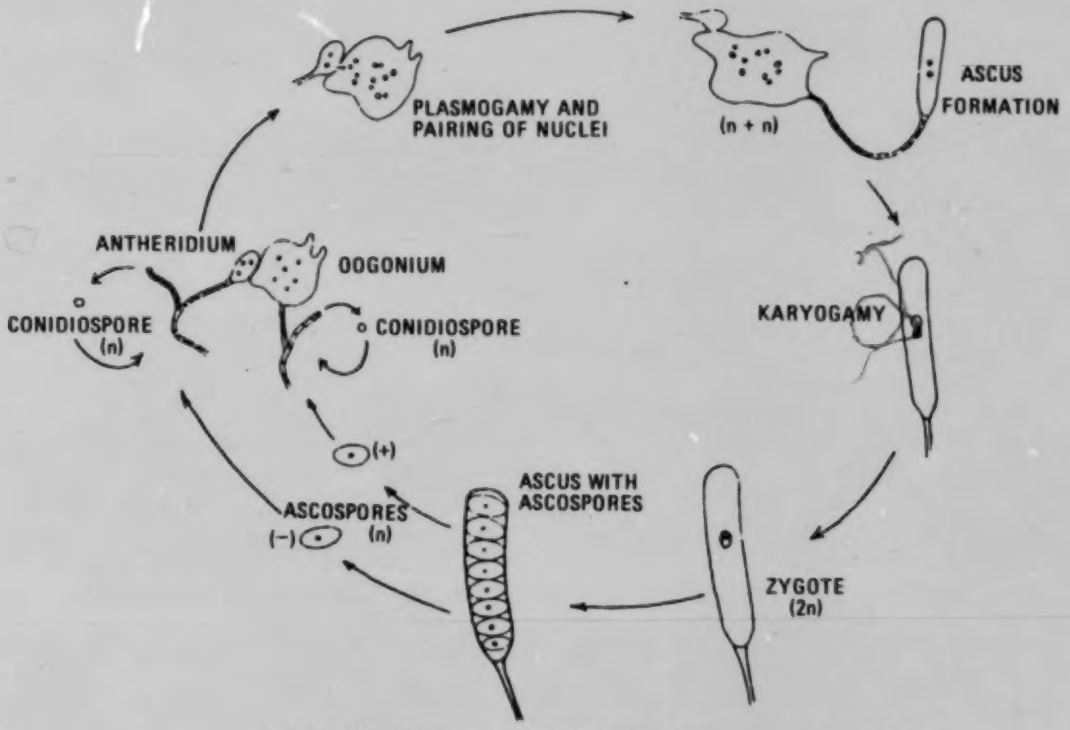
Study a living culture of yeast and note that they can reproduce vegetatively by budding.

Study material from the reproductive cycle. Note: the hyphae producing the conidiospores are haploid. When male (–) and female (+) hyphae are in close association, specialized sex structures are formed. The (–) hyphae form a spermatogonium (also called an antheridium), and the (+) hyphae form an oogonium (also called an ascogonium). A fusion of these two sexual structures permits the nuclei from the spermatogonium to enter the oogonium. The two types of nuclei do *not* fuse at this time. Thus, we say plasmogamy (cytoplasmic fusion) has occurred but not fertilization (the fusion of male and female nuclei).

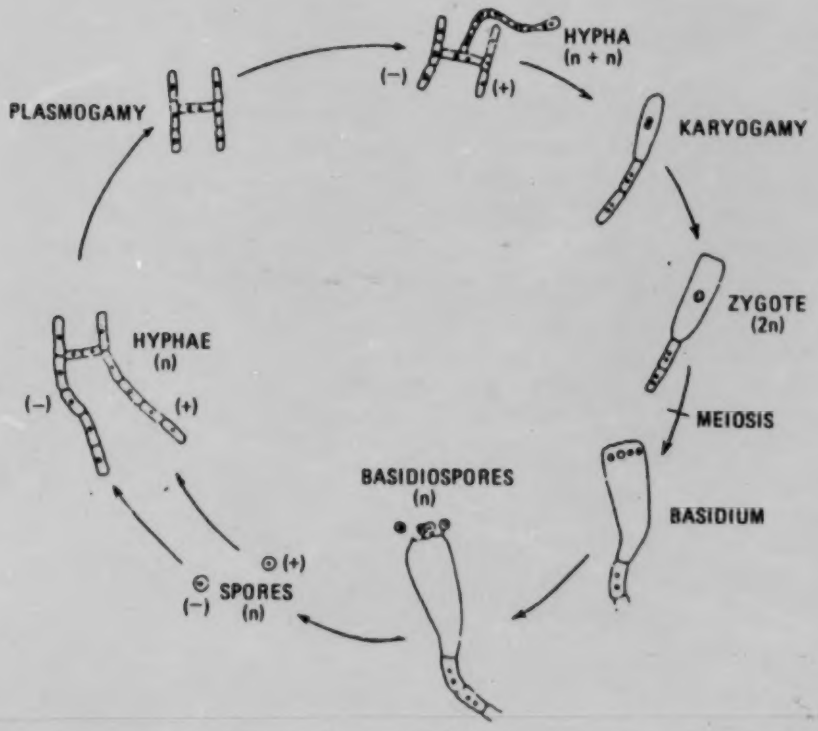
Branch hyphae develop from the oogonia. Each cell of such hyphae contain a male nucleus and a female nucleus, which are diploid and thus represent the diplo- phase of the life cycle. Later the terminal cell enlarges and the two nuclei fuse (karyogamy). The zygote thus formed undergoes meiosis resulting in eight haploid ascospores within the sac-like ascus. It is not infrequently seen that the hyphae may grow around the ascus to form the finished fruiting body called an ascocarp.

5. Basidiomycetes

The Basidiomycetes include the mushrooms, shelf fungi, the rusts and the smuts of grains. In mushrooms the haploid hyphae fuse by plasmogamy to produce diploid hyphae which will in turn form the fruiting body or basidiocarp, the mushroom body. The terminal cells (basidia) of the diploid hyphae arrange themselves on the margins of the gills. Karyogamy occurs, followed by meiosis which produces four basidiospores.




Life Cycle of an Ascomycete Like *Neurospora Crassa*




Life Cycle Among the Mushrooms (Basidiomycetes)

## REPORT SHEETS FOR EXERCISE 20

### Vegetative and Sexual Reproduction

Questions raised in the Introduction:

1. How would you describe the number of chromosomes produced by mitosis in the juvenile or adult forms in these reproductive patterns?

_____ Haplontic
_____ Diplontic
_____ Haplodiplontic

2. What is the number of chromosomes in the cells of the adult organism reproducing in the

_____ Haplontic pattern?
_____ Diplontic pattern?

3. What is the number of chromosomes in the cells of these stages of the diplontic pattern?

_____ Sporophyte
_____ Gametophyte

Identify or define these terms:

4. Gamete

5. Fertilization

6. Haploid

7. Diploid

8. Mitosis

9. Meiosis

10. Meiospore

**Vegetative and Sexual Reproduction** **Part** _____

1. Below is depicted stages in the life cycle of _____,
   which was studied from _____ specimens.
   (LIVING OR PRESERVED?)
   The haploid stages are indicated by n and the diploid stages by 2n. All stages are named.

**Vegetative and Sexual Reproduction** **Part _____**

In the space below write a report on the ecology, economic importance and reproduction in the organisms whose life cycle you studied and depicted on the previous page.

See what other members of the class found out about the life cycles of other organisms in this exercise.

Answer these general questions.

1. What are the advantages of vegetative reproduction?

2. What are the advantages of sexual reproduction from a biological point of view?

# EXERCISE 21 – REPRODUCTIVE STRUCTURES IN FLOWERING PLANTS (ANGIOSPERMS)

Materials and Equipment

Sweet potato
Roots of a sweet potato plant with young potatoes (demonstration)
Sweet potato soaked in water or planted for several weeks
Corn plants several weeks old with suckers
Irish potato
Irish potato soaked or planted for several weeks
Pots of Hens and Chickens, Strawberry plants or Bermuda grass
Potted saplings of woody plants
Sharp knives
Soft paraffin
Blossoms of lily, and sweet pea
Preserved early ears of corn and preserved (dry) tassel
Onions

## Part A. Vegetative Reproduction (Without Respect to Sex)

1. Natural Vegetative Reproduction

a. *Rooting of Above-Ground Stems.* Some plants send out stems extending over the ground. There these take root at specific places (where?), sending up leafy shoots and send down into the soil a system of roots and rootlets. These stems are called runners and stolons.

Examine a pot of Hens and Chickens, Strawberry plants or a pot of Bermuda grass.

b. Other plants send out stems underground, sometimes for considerable distances. These stems are called rhizomes. How do they differ from the roots? As was true for the above-ground stolons, note that the roots and shoots occur at characteristic points.

Examine the root system of an Irish potato plant. Identify rhizomes and roots. Where are the potatoes forming?

Examine an Irish potato. Look at the eyes with a lens. What is an eye? Sketch one. True roots formed along stems and in other unusual places (like at the base of leaf petioles) are called adventitious.

c. Sometimes new plants arise from the true roots of parent plants. Examine the roots of a sweet potato plant. Do you see stolons? Do sweet potatoes have the same kind of eyes as Irish potatoes? Examine under a lens and sketch one. Examine a corn plant several weeks old which has suckers. Do they arise from the stem or roots?

d. Bulbs and Corms are enlargements of leaves and stems respectively. Examine an onion bulb. Find the root and radicle. Compare with the corm of gladiolus or of crocus.

2. Artificial Asexual Reproduction

A Washington Elm grows on the Cambridge, Massachusetts common. Some historians of the tree say that this one (1970) is a grandchild of the original tree which was destroyed in a storm. A branch from that tree had been rooted to form the second Washington Elm, which finally succumbed, but before it did a cutting was taken and rooted to form the present tree. Are these different trees or the same tree?

Many plants are propagated by either taking cuttings and rooting them, or by a process called "layering" by nursery men. This consists in bending a plant over to the ground and covering with soil. When the sprouts are large enough, the parent stem is cut up and the new plants potted or transplanted to the growing field. Such a group of plants derived asexually from a single parent is called a clone.

3. Grafting and Budding

There are a variety of patterns for cutting twigs for grafting. The essential thing is to establish good contact with the cambium layers of both the stock (which receives the graft) and the scion (which is the piece grafted on).

a. On a plant label write your names and the date. Attach to a stock twig on the sapling. Cut the twig off a foot or so from the end. Exchange this piece with another group.

b. Cut the bottom of the scion into a wedge at the cut end.

c. Split the stock so that it will receive the scion. Match up the green layers of the cambium. Bind the stock firmly with twine and enclose the cut areas with soft paraffin (Figure 1).

d. Cut under a bud or small twiglet.

e. Make a T-shaped cut in the bark of the stock.

f. Insert the bud into the incision, being sure that there is contact between the cambium layers of the scion and the stock.

g. Bind firmly with twine. Apply some soft paraffin to the cuts to prevent too much evaporation but do not cover the bud (Figure 2).

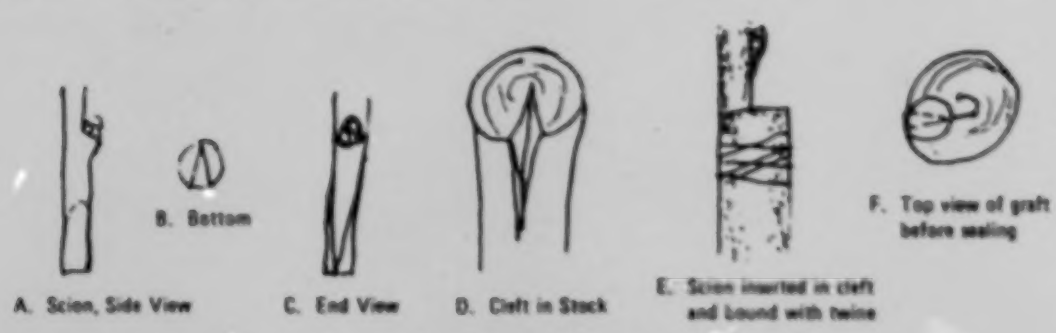

B. Bottom
F. Top view of graft before sealing
A. Scion, Side View
C. End View
D. Cleft in Stock
E. Scion inserted in cleft and bound with twine

Figure 1. Cleft Grating

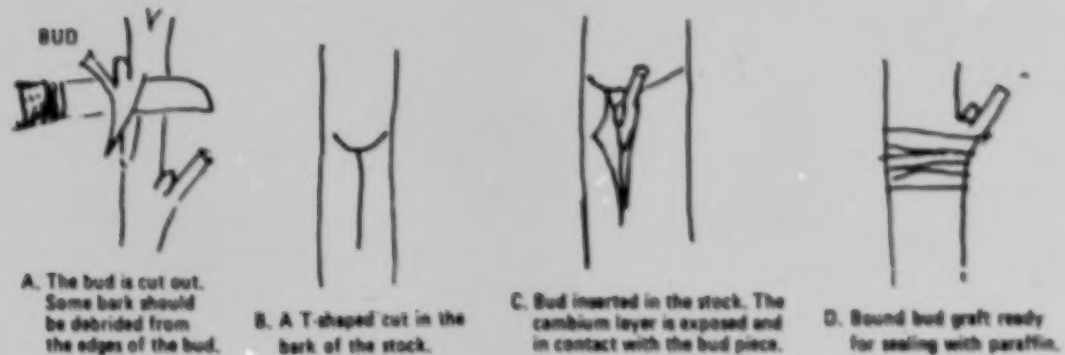

BUD
A. The bud is cut out. Some bark should be debrided from the edges of the bud.
B. A T-shaped cut in the bark of the stock.
C. Bud inserted in the stock. The cambium layer is exposed and in contact with the bud piece.
D. Bound bud graft ready for sealing with paraffin.

Figure 2. Budding

**Part B. The Structure of Flowers**

General design of a flower is shown in Figure 3. Flowers vary a great deal as to which parts are emphasized or repressed. Some will be monecious, that is, having only pistils (piltillate flowers) or stamens (staminate flowers) among their reproductive parts.

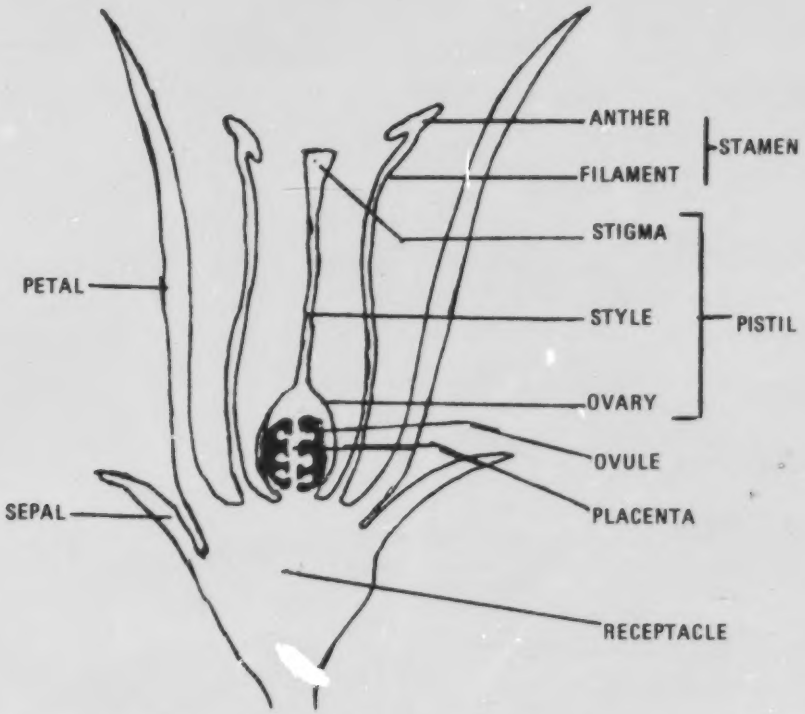



Figure 3. Diagram Showing the Parts of a Flower (In General)
Sagittal Section

1. Examine a lily and identify all of the parts in the diagram. How many of each part are there? How are they arranged?

2. Examine a flower of the sweet pea. It is very similar to that of the bean but is larger and therefore easier to study. The petals are somewhat modified. The standards are the largest, forming the back of the flower. The wings lie between the standard and the keel. All of these are attached to a receptacle which is within a calyx made of sepals.

Remove the keel, revealing the reproductive structure beneath (within) it. Remove the standard and wing petals on one side and sketch the positions of the pistils and stamens in relation to the rest of the flower.

3. Examine a fresh or preserved specimen of a young ear of corn. Identify the stigmas, styles and ovaries of the flowers. Where are the petals, sepals and stamens? Look at some silk under the microscope.

Now look over a corn tassel carefully. Examine one of the pendants of the tassel under the microscope. Where are the sepals, pistils, stamens, and petals?

## REPORT SHEET FOR EXERCISE 21

### Part A. Vegetative Reproduction

Sketch of main plant with stolon and secondary plant.

Eye of an Irish potato drawn from magnification _____X

What part of the plant is the Irish potato?

Eye of a sweet potato drawn from magnification _____X

What part of the plant is the sweet potato?

Sketch a cross section of an onion bulb.

Sketch of a cross section of the corm of

Of what advantage is it to a flowering plant to be able to reproduce vegetatively?

**Part B. The Structure of Flowers**

Diagram of a lily blossom seen from the top. (Label all parts.)

Sweet pea blossom sketched from the side after removing petals from one side. (Label all parts.)

Flowers of the female corn plant. Male

What is meant by monecious and dioecious?

# EXERCISE 22 – SEED AND FRUIT PRODUCTION IN FLOWERING PLANTS

The flower forms on the sporophyte in the life cycle of flowering plants. The "spores" produced are the female megaspore, which will give rise to the female gametophyte, and the male microspores, which develop in the microsporangia of the anther. The spore mother cells are diploid, but undergo meiosis to yield four haploid cells (as in animals). In the case of the male, the four microspores formed stay grouped together in a tetrad. It takes one more division to convert microspores into pollen grains. Each of the four spores in the tetrad undergo a nuclear division (not cytoplasmic) resulting in two nuclei in each cell. These are differentiated so that one becomes the generative cell (sperm nucleus) and the other will become the cell which will extend from the top of the stigma on the pistil down into the ovule of the female gametophyte. The sperm nucleus divides once to form two gamete nuclei.

In the female part (ovule) the megaspore mother cell undergoes meiosis without cytoplasmic division, yielding four nuclei in a line. Three of these disintegrate–the one furthest from the micropyle and nearest the source of nourishment survives. The megaspore then divides 3 times to form the embryo sac, with 2 groups of 4 nuclei. One of each group, the polar nuclei, migrate to the center of the sac, and one, near the micropyle, becomes the egg. The rest of the nuclei become functionless.

When the pollen tube enters the micropyle, it releases its two sperm nuclei. One of these combines with the egg cell nucleus to form the zygote and the other fuses with the two polar nuclei in the center of the sac to form the triploid nucleus of the primary endosperm cell. Thus, in flowers there is double fertilization resulting in a diploid zygote of the next sporophyte generation and the triploid endosperm cell for gathering food to feed the embryo during its early growth from seed. The forms of the matured ovary and its protective coverings and food supplies which constitute the seeds and other parts of the fruit we shall inquire into in this exercise.

## Part A. Germination of Pollen Grains

Materials and Equipment

A supply of pollen or flowers with ripe anthers
10%, 20%, 30%, 40%, and 50% (weight/weight) solutions of sucrose, glucose or honey (about 10 ml. for the class)
5%, 10% and 20% sodium chloride solutions
Indoleacetic acid (100 mg./100 ml.)
Gibberellic acid (100 mg./100 ml.)
Pasteur pipettes
Microscope slides and coverglasses
Petrolatum (e.g., Vaseline) warmed just to melting on a controlled heat hot plate
Small camel hair brushes (like used for handling fruit flies)
Microscope and lamp
Wax pencil

Procedure

1. Label 18 microscope slides 1 to 18 with wax pencil.

2. Add a few pollen grains to the center of each slide.

3. Using a separate Pasteur pipette for each solution, add a small drop of liquid to each slide. For comparisons with the rest of the class use this series below. Remember, where 2 drops of different solutions are used the concentrations are halved.

| SLIDE | SOLUTION ADDED TO POLLEN | SLIDE | SOLUTION ADDED TO POLLEN | SLIDE | SOLUTION ADDED TO POLLEN |
|---|---|---|---|---|---|
| 1 | water | 7 | water + hormone* | 13 | 5% salt |
| 2 | 10% sugar* | 8 | 10% sugar + hormone | 14 | 10% salt |
| 3 | 20% sugar | 9 | 20% sugar + hormone | 15 | 20% salt |
| 4 | 30% sugar | 10 | 30% sugar + hormone | 16 | 5% salt + hormone |
| 5 | 40% sugar | 11 | 40% sugar + hormone | 17 | 10% salt + hormone |
| 6 | 50% sugar | 12 | 50% sugar + hormone | 18 | 20% salt + hormone |

*As assigned by the instructor

Note: Because NaCl dissociates, it will provide almost twice as many particles in solution as the same amount of sugar and therefore 5% NaCl will approximate 10% sugar, 20% NaCl approximates the osmotic activity of 40% sugar, etc.

4. Mix the material on each slide with the end of a fresh toothpick or piece of applicator stick. Cover with a coverglass and seal by applying melted petrolatum to the edges of the coverglass using a camel hair brush. Check all of the slides under high power of the microscope at this time and from time to time during the laboratory period.

**Part B. The Kinds of Fruits**

Inspect the types available in the laboratory and complete the proper Report Sheet.

1. Simple Fruits

a. Simple Dry Fruits

| TYPE | FRUIT OF |
|---|---|
| Follicle | Milkweed |
| Capsule | Okra |
| | Cotton |
| Legume (Pód) | Bean |
| | Green Pea |
| Achene | Dandelion |
| | Sunflower |
| | Strawberry |
| Grain | Corn |
| | Wheat |
| Samara | Ash, Elm or Maple |
| Nuts | Acorn |
| | English walnut (with brachts) |
| | Black walnut (with brachts) |

b. Simple Flesh Fruits

| TYPE | FRUIT OF |
|---|---|
| Berry | Tomato |
| | Banana |
| | Avocado |
| | Eggplant |
| Drupe | Peach |
| | Plum |
| Fibrous Drupe | Coconut |
| Pome | Apple |
| | Quince |

2. Aggregate Fruits

| | |
|---|---|
| Drupelets | Raspberry (receptacle usually dry) |
| Aggregate-accessory | Blackberry (receptacle becomes fleshy supporting the drupelets) |
| | Strawberry (receptacle becomes fleshy supporting achenes) |

3. Multiple Fruits

Pineapple
Osage Orange

## REPORT SHEETS FOR EXERCISE 22

### Part A. Germination of Pollen

GERMINATION OF THE POLLEN GRAINS OF _____ AFTER _____ MINUTES IN VARIOUS MEDIA

| Slide No. | Approximate % Germination | Tube Growth* | Slide No. | Approximate % Germination | Tube Growth* |
|---|---|---|---|---|---|
| 1 | | | 11 | | |
| 2 | | | 12 | | |
| 3 | | | 13 | | |
| 4 | | | 14 | | |
| 5 | | | 15 | | |
| 6 | | | 16 | | |
| 7 | | | 17 | | |
| 8 | | | 18 | | |
| 9 | | | | | |
| 10 | | | | | |

* 0 = none; + = emerging; ++ = some growth; +++ = much growth

1. Is this pollen highly selective of its germinating medium?

2. Does it appear that germination is a function of water (hydration) or of dehydration (exosmosis)?

3. Interpret the results that you obtained in this experiment.

**Part B. The Kinds of Fruits**

1. a. Simple Dry Fruits

| TYPE | FRUIT OF | DEHISCENT? | HOW SPREAD? |
|---|---|---|---|
| Follicle | Milkweed | | |
| Capsule | Okra | | |
| | Cotton | | |
| Follicle | Milkweed | | |
| | Okra | | |
| Capsule | Cotton | | |
| Legume (Pod) | Bean | | |
| | Green Pea | | |
| Achene | Dandelion | | |
| | Sunflower | | |
| | Strawberry | | |
| Grain | Corn | | |
| | Wheat | | |
| Samara | Ash, Elm or Maple | | |
| Nuts | Acorn | | |
| | English Walnut | | |
| | Black Walnut | | |

1. b. Simple Fleshy Fruits

| | | What part is mainly eaten? (Check) | | | | |
|---|---|---|---|---|---|---|
| | | PLACENTA | PERICARP | ENDOCARP | MESOCARP | EXOCARP |
| Berry | Tomato | | | | | |
| | Banana | | | | | |
| | Avocado | | | | | |
| | Eggplant | | | | | |
| Drupe | Peach | | | | | |
| | Plum | | | | | |
| Fibrous Drupe | Coconut | | | | | |
| Pome | Apple | | | | | |
| | Quince | | | | | |

2. Aggregate Fruits

| | | What part is mainly eaten? (Check) | |
|---|---|---|---|
| | | PERICARP | RECEPTACLE |
| Drupelets | Raspberry | | |
| Aggregate- | Blackberry | | |
| accessory | Strawberry | | |

3. Multiple Fruits

Pineapple

Osage Orange

# EXERCISE 23 – MONOCOT AND DICOT SEEDS, SEEDLINGS AND LEAVES

Material and Equipment

Hand lens or dissecting microscope
Bean and corn seeds soaked overnight
Bean and corn seedlings (Seeds are placed between cotton and the sides of a jar. The cotton is wet and watered daily and the jar is kept in a dark cabinet for a few days. After the seedlings have developed leaves they may be left in the light.
Prepared slide of monocot and dicot leaf cross sections

**Part A. Seeds** (Combined with Report Sheet)

1. Examine a corn seed that has been soaked for about 24 hours. On one side note the region of the embryo indicated by a whitish scar and just above it the wrinkled silk scar. The seed had been attached to the cob by its hilum. The outer covering is called the testa, or fruit coat.

2. With a sharp scalpel or razor blade cut the seed longitudinally through the region of the embryo. Note that the starchy endosperm can be divided into the soft inner starch endosperm and the outer yellowish horny endosperm.

3. The single cotyledon of the seed lies between the endosperm and the embryo. The upper part of the embryo or epicotyl consists of the plumule and coleoptile (or plumule sheath). The lower part or hypocotyl contains the radicle with its root cap.

4. Draw this sagittal section through the seed in the space at the right. Label the parts.

5. Examine a bean seed that has been soaked overnight. On one side note the hilum and near it the minute opening called the micropyle. On the side of the hilum opposite the micropyle find the chalaza, a small protuberance where the various integuments meet to form the seed coat.

6. Remove the testa with a scalpel or needle. Beneath the hilum note the pointed radicle. Separate the two halves (each a cotyledon or seed leaf) and examine with a lens. In the embryo note the plumule which already contains veined leaves, and the radicle.

7. Draw the half of the seed containing the embryo in the space at the right.

**Part B. Seedlings**

1. Examine a corn seedling which has been allowed to sprout and grow in cotton. Remove the cotton.

2. Observe the young root with its root hairs, the young shoot, the cotyledons and the leaves.

3. Draw the seedling in the space at the right.

4. Examine a bean seedling which has been allowed to sprout and grow against the side of a jar, held in place by cotton. Remove the cotton.

5. Observe the young root with its root hairs, the cotyledons and the young shoot.

6. Draw the seedling in the space at the right.

**Part C. Leaves**

1. Draw a leaf of the corn seedling, paying attention to the pattern of the veins.

2. Draw a leaf from a dicotyledonous plant, such as the bean. Identify the margin, veins, lamina and petiole.

**Part D. Leaf Cross-sections**

1. Study a prepared slide of cross sections through a monocot leaf. Make a drawing through a portion showing a vascular bundle and if possible a stoma.

2. Make a drawing of a dicot leaf including a vascular bundle and if possible a stoma. Label the cuticle, upper epidermis, palisade cells, air spaces, spongy layer, a vein containing xylem and phloem, and the lower epidermis.

# EXERCISE 24 – HISTOLOGICAL STUDY OF THE FEMALE REPRODUCTIVE SYSTEM

## Materials and Equipment

Microscope and lamp
Immersion oil and lens paper
Prepared slides of the following:
- Rat, mouse, rabbit or human ovary
- Oviduct
- Uterus .

## Introduction

The female genital system includes the ovaries, oviducts, the uterus with its cervix (neck), vagina (sheath) and some external folds, the vulva. The ovogonia develop in the ovary within a group of granular cells, the granulosa. In its early stages as a "primordial follicle" growth does not depend upon pituitary gonadotropins. When an ovogonium becomes privileged to develop (primary ovocyte), LH from the anterior pituitary gland acts to start the antrum of the Graafian follicle, after which FSH takes over the growth-stimulation. When the follicle is large and the antrum filled with fluid, the process of luteinization begins under the influence of LH. The conversion of the granulosa cells which secrete estrogens (particularly estradiol, and estrone) to their luteinized form makes them responsive to a third pituitary hormone, luteotrophin or prolactin, which then causes these cells to secrete progesterone, a hormone needed for the maintenance of pregnancy. The estrogens bring about growth of the reproductive tubes, which have been captured from the urinary system. Following ovulation, which occurs accidentally during luteinization of the follicle, the progesterone converts the enlarged tubes into a suitable environment for the development of embryos. If pregnancy does not occur, the mucosa will return to the estrogenized state except among the higher primates and women where the mucosa breaks down due to the withdrawal of hormonal support, and becomes quite thin before being again stimulated to growth by estrogenic hormones secreted by new developing Graafian follicles. This cycle of events, which is characterized by estrus or "heat" at ovulation time among the lower mammals, is called an estrous cycle and lasts about 5 days in rats and mice, 16 days in guinea pigs, 3 weeks for sheep and swine. Rabbits will remain in almost "constant estrus" until mating occurs due to the consecutive maturation of follicles, but most domestic animals–horses, cows, dogs and cats, will have a 3 week cycle about every 6 months. Since the breakdown of the mucosa in the higher primates is accompanied by bleeding, and since there are no periods of "heat," the sex cycle is dated from the onset of menstruation (bleeding) and is called a menstrual cycle. This lasts about 27 to 32 days in Macaque monkeys and in women and about 35 days in baboons. In this exercise we will study some organs of the female reproductive tract as they pass through a non-pregnant cycle.

## Procedure

### A. The Ovary

The ovary in rats and mice are small, mulberry-shaped bodies 3-6 mm. in diameter. The ovaries of the rabbit are elongated organs about 5 by 10-15 mm. The human ovary is about the size and shape of a large almond–about 1.5 to 3.0 cm. in width and 2.5 to 5.0 cm. in length. Study a section of an ovary and observe these structures:

1. *Germinal epithelium.* The free surface of the embryonic ovary is covered by cuboidal epithelium, which becomes thinner with age so that it is stretched thin or absent in the adult.

2. *Cortex and medulla.* The ovary is divided into a *medulla* and a *cortex* of connective tissue, developing and mature follicles, corpora lutea and interstitial tissue which is probably the residue of atretic follicles, and blood vessels. The cortex is divided from the inner medulla by a thin layer of condensed connective tissue, the *tunica albuginea*. Beneath this lies a few coiled tubules of the *rete ovarii*, a vestige of its connection with the embryonic mesonephros.

3. *Follicles.* Make a *drawing* of a primordial follicle, a primary follicle and a mature Graafian follicle.

a. A primordial follicle has an *ovogonium* at its center. This is characterized by a large nucleus, usually eccentrically located. The germ cell is surrounded by a layer of *granular cells* ranging from flattened to columnar in height and is bounded on the outside by a basement membrane, separating the follicle from the *interstitial connective tissue*.

b. A primary follicle will have between 15 and 20 layers of *granulosa cells* and a small cleft within the granulosa, the *antrum*. The ovocyte is separated from the granulosa by a non-cellular *zona pellucida*. Beyond the basement membrane of the granulosa the surrounding interstitial stroma has become organized into an investing layer, the *theca folliculi* which is richly supplied with *blood vessels*.

c. The mature Graafian follicle is much larger than a primary follicle. The *antrum* is filled with *follicular liquor*. The granulosa is heaped up around the *ovocyte* to form the *cumulus oophorus* which may be vacuolated across its base. Beyond the *basement membrane* the theca is divided into two parts, the more definite *theca interna* and the outer, more loosely organized *theca externa*. These two layers are separated by a stratum of *blood vessels*. It is the theca interna that, under the influence of FSH, secretes estrogens that are carried by the blood but also accumulate in the follicular liquor.

4. *Corpora lutea.* Following the action of LH, the granulosa cells become swollen and epithelioid and are pushed into the old antrum by infoldings of the *theca interna*. Thus two types of lutein cells are distinguished, *thecal lutein cells* and *true lutein cells* developed from the granulosa. Many blood vessels are to be seen in the corpus luteum parenchyma. The corpus luteum is then bounded by the old *theca externa*. In rat and mouse ovaries the corpora lutea protrude from the ovarian surface while in the ovaries of rabbits and women they are contained within the body of the gland.

B. The Oviduct (Fallopian Tube)

The oviduct receives the ovulated egg either directly from the ovary or from the body cavity. In rodents the ovary is usually surrounded by a periovarial sac to reduce the chance of fertilized eggs getting lost in the peritoneum. It is a much-coiled tubule which will store the ovum for 4 to 5 days before permitting it into the uterus. During this time the uterine lining is becoming more glandular in preparation for the blastocyst. If the egg was fertilized, it will enter and implant, if it was not it will die and be reabsorbed before the tubo-uterine sphincter opens under the influence of this prolonged progestational stimulation. Look for the ciliated columnar or ciliated pseudostratified columnar epithelium lining the lumen.

C. The Uterus

The uterus consists of an endometrium or mucosa containing endometrial glands, and a myometrium, consisting of an inner circular and an outer longitudinal muscularis. The whole is bounded on the outside by a serosa. The height of the endometrium and the state of its glands depends upon the stage of the sex cycle. Estrogens increase the height of the endometrium but leave the glands relatively straight. Under the influence of progesterone, the grown-up epithelium does not grow much more, but the glands become highly coiled and distended with secretion.

## REPORT SHEET FOR EXERCISE 24

**Histological Study of the Female Reproductive System**

3a. A primordial follicle in the ovary of a _____
Drawn from study at a magnification of _____X

3b. A primary follicle in the ovary of a _____
Drawn from study at a magnification of _____X

3c. A mature Graafian follicle in the ovary of a _____
Drawn from study at a magnification of _____X

# EXERCISE 25 – HISTOLOGICAL STUDY OF THE MALE REPRODUCTIVE SYSTEM

## Materials and Equipment

Microscope and Lamp
Lens paper and immersion oil
Prepared slides of the following:
Mammalian testis, Ductus deferens, seminal vesicle and prostate gland

## Procedure

The testes, epididymis, ductus deferens, urethra, and penis, together with their associated glands and other structures make up the male reproductive system.

### A. The Testis

The testes are the paired gonads of the male and in most mammals they are suspended in an extension of the paritoneal cavity, the cavita serosa or male vagina. This cavity is lined by a serous membrane forming the tunica albuginea on the testicular side and the tunica vaginalis propria next to the muscular wall of the scrotal sac. In mammals the testes are mixed glands. The exocrine portion is cytogenic and "secretes" gametes (sperm) and the endocrine portion secretes androgenic hormones, chiefly testosterone.

1. *General Structure.* Study a cross section of the testis (slide No. ). Note that the gland is surrounded by a white connective tissue capsule, the *tunica albuginea.* Within this capsule cross sections of the compound ramifications of the *seminiferous tubules* are seen and between these tubules there are numerous *blood vessels* and the epithelioid cells of the *interstitial tissue* (Leydig cells). The tubules are almost filled by the complex *seminiferous epithelium* which rests on a distinct *basement membrane.*

*Draw* in outline 3 or 4 tubules next to the tunica albuginea, indicating the lumina and basement membranes but omitting the details of the seminiferous epithelium. Make your drawing large enough so that the *details* of the interstitial cells can be clearly shown. The interstitial cells respond to the luteinizing hormone (LH) of the anterior pituitary gland by secreting androgens (male sex hormones) and a few other steroids in smaller amounts. Note that the blood vessels tend to course through the interstitial tissue rather than next to the basement membranes. Why?

2. *Tubular Structure.* Study several tubules under high power or under oil immersion. Select a tubule which shows a variety of cell types in the seminiferous epithelium. *Draw* a section from the lumen to the basement membrane. The most basal layer will contain two types of cells. The *spermatogonia* are small cells with deep-staining nuclei. The Sertoli (sustentacular) *cell* nuclei are usually triangular or irregularly shaped, light-staining, vesicular bodies with the cytoplasmic portion of the cell extending to or near the lumen. The cytoplasmic portion of the Sertoli cells are easily seen in those tubule sections where most of the sperm have matured and have been released. In the second layer of the seminiferous epithelium the *primary spermatocytes* have undergone their growth phase and are about twice the size of spermatogonia. The nuclei are large and in various stages of prophase. The next layers of the epithelium will have perhaps only one or two of the following cell types: dividing *primary spermatocytes, secondary spermatocytes* (which are about the size of the spermatogonia) and *spermatids* (which have vesicular, spherical, pale-staining nuclei and a distinct centriole) and *metamorphosing spermia* and *matured sperm* attached to Sertoli cells. The tails of the mature spermia are seen as whorling masses of filaments in the tubule lumena. What is the "spermatogenic wave"?

B. The Ductus Deferens (Vas Deferens)

Study a cross section of the ductus deferens (slide No. ). Note that its mucosa consists of *pseudostratified columnar epithelium* which may have stereocilia and is thrown into longitudinal folds. Beneath the distinct *basement membrane* is a thin *lamina propria* of elastic tissue. The *muscularis* consists of three layers, an *internal longitudinal layer*, a thick *middle circular layer*, and an *external longitudinal layer* (compare with the lower ureter). The *adventitia* consists of connective tissue with accompanying blood vessels and nerves. Make an outline *drawing* of the ductus deferens and label the layers.

C. The Seminal Vesicles

These organs are evaginations of the ductus deferens although their organization differs slightly from the ductus deferens. They are elongate structures with a mucosa that branches and anastomoses so that it contains a number of acini or pockets.

*Study* a frontal section through the seminal vesicles (slide No. ). Note the connective tissue *capsule* which envelopes the *musculo-connective tissue* which is made up of smooth muscle, reticular cells and dense connective tissue. There is no muscularis mucosae separating the musculo-connective tissue and the *lamina propria*, but the latter tissue can be distinguished because it stains more deeply and is filled with a prolific *capillary network*. There is no basement membrane under the *epithelium*, only a felt-work of connective tissue fibers. The epithelium is of the simple columnar type in rodents but is pseudostratified in man and may even be ciliated. Hematoxylin and eosin preparations show *"secretion granules"* in the cytoplasm. The height of the epithelium and the number of secretion granules depend upon the functional state of the seminal vesicles as regulated by hormones from the testes and adrenal glands. *Draw* 2 or 3 epithelial cells showing all details.

D. The Prostate Gland

Study a section through the ventral prostate of the rat (slide No. ). Note that this is a compound tubular type gland. The tubules are embedded in *areolar connective* tissue which contains a number of Mast cells. The glandular portion of the epithelium is formed as secretory alveoli in the peripheral part (cortex) of the gland. These alveoli are continuous with the secretory ducts which have low cuboidal epithelium and are rather straight. In prostate tissue from middle-aged men one can see concretions occluding the lumens. Lamination of these concretions (corpora amylacea) is not always evident in sections. They are not observed in rat material. *Draw* an alveolus with some of its surrounding connective tissue.

## REPORT SHEET FOR EXERCISE 25

**Histological Study of the Male Reproductive System**

1. Interstitial cells between the tubules of the testis of the _____
   Drawn from study at a magnification of _____X

2. The germinal epithelium showing the development of spermatozoa from spermatogonia as studied in the testis of _____.
   Drawn from a magnification of _____X

# EXERCISE 26 – RESPONSE OF ANIMALS TO PREGNANCY URINE HORMONE

## Pregnancy Tests

### Materials and Equipment

Pregnancy urine (preferably from the 4th to the 12th week of gestation)
Urinometer (for determining specific gravity)
20% HCl
.04% Bromcresol green indicator dye
Kaolin (acid washed)
.1N NaOH
.5% Phenolphthalein
1% eosin blue or yellow
4% aniline blue -
Citrate-phosphate buffer, pH 7.2
pHydrion paper

### Cutler's[1] Method for Concentrating Pregnancy Urine Hormone (PU)

Use 100 ml. of normal urine of pregnancy (sp. gr. 1.010) or 50 ml. if the specific gravity is 1.015 or higher diluted to 100 ml. Acidify to pH 4 using 20% HCl with 0.04% bromcresol green as an indicator. Then add 5 cc. of a 20% water suspension of kaolin that has been acid washed. (See Hawk and Gergeim, 11th ed., pg. 263, *Practical Physiol. Chem.*)

Shake and place in the cold room for 30 minutes or until the kaolin has settled down to 15 to 20 ml., then siphon off the supernatant. Redistribute the packed kaolin in 5 cc. of N/10 NaOH. Allow to settle. Decant supernatant and make pink with a drop of 0.5% alcoholic phenolphthalein. Neutralize with 20% HCl.

Inject 1 cc. into the dorsal lymph sac of R. pipiens. In case the patient may be in menopause, use rabbit or African frog (Xenopus laevis), or 2½ cc. of whole urine injected into R. pipiens at hourly intervals.

Male frogs will usually release spermatozoa with this preparation in 30 to 120 minutes.

### Vital Staining of Spermatozoa

Various concentrations of dyes and buffers at different pHs were tested by Shaffer and Almquist with the following giving the most satisfactory results for distinguishing live and dead spermatozoa in bull semen:

1% eosin blue (or yellow)
4% aniline blue
Citrate-phosphate buffer, pH 7.2

This has not been modified for use with wet frog smears. Perhaps 2 drops of buffer and 1 drop each of the stains, mixed on the slide and air-dried will give satisfactory results.

**Reference:**

Shaffer, H. E. and J. O. Almquist. Journ. Dairy Sci. 31: (8): 677–678, 1948, p. 36 (abstracted in Section III, Excerpta Medica 3 (9): 362, No. 1400, 1949)

[1] Cutler, J. N., Jour. Lab. and Clinical Med., 34:544–559, 1949.

Procedure

*Aschheim-Zondek Test* – AZ Test:

The method employs the high titre of "LH"–really Chorionic Gonadotropin, "PU"–present in pregnancy urine. The ovaries of immature mice are ordinarily small and quiescent, but under the stimulation of PU they ovulate and develop corpora hemmorrhagica and/or corpora lutea. A positive is obtained when a single corpus or more is discovered.

Method is as follows:

1. Take a morning sample of urine and add one drop of Toluene per 30 ml. of urine. Acidify slightly with a few drops of 10% acetic acid. Store in cold room when not in use. However, it is better to eliminate toxins using the method of Cutler (above).

2. Use 6 immature white mice (10 grams body weight) for each urine sample to be tested. The mice are used only once.

3. Inject twice a day for three days via the subcutaneous (s/c) route according to this modification:

Mouse 1 and 2 uninjected controls
Mouse 3–inject 0.2 ml. urine s/c 2X daily for 3 days
Mouse 4–inject 0.25 ml.
Mouse 5–inject 0.3 ml.
Mouse 6–inject 0.4 ml.

4. Kill all mice and examine the ovaries under the dissecting microscope for corpora 96 hours (four days) after the first injection.

5. A single corpus means a positive result. Record the results in your notebook. Pay particular attention to the color of the ovaries as compared to the controls.

*Rana Pipiens Test:*

Mammalian gonadotropins have been demonstrated to affect the reproductive systems of amphibia. The induction of spermatogenesis (rather release of sperm) by chorionic gonadotropins is used for a positive diagnosis of pregnancy. It is to be noted that seasonal effects are important, for the frog test does not work accurately in the late summer months. We shall use the method of Robbins:

1. Filter an aliquot of morning urine. No other treatment is necessary.

2. Take control cloacal smears of your male frogs to insure they are not releasing sperm. Smears may be read under reduced light in a microscope, or you may fix and stain them with Giemsa stain in the same way you prepare vaginal smears for study.

3. Inject 4 to 5 ml. of urine into the dorsal lymph sac of each of four frogs. Four frogs are used today to overcome the possible fatalities due to the toxicity of the urine. Test each sample of urine. Only one frog is needed if concentrated PU is used.

4. Two hours after the injection take cloacal smears and examine them for sperm.

5. A positive result is indicated when sperm are detected.

**References:**

For AZ Test: Aschheim, S., and B. Zondek, 1927. Ei und Hormon, Klin. Wehnschr. 6:1321.
For Rana Pipiens Test: Robbins, S. L. and F. Parker. The use of the male North American frog in the diagnosis of pregnancy. Endocrinology 42:237 (1948).
General: Hoffman, J. *Female Endocrinology*, p. 670 ff.

## REPORT SHEET FOR EXERCISE 26

**Pregnancy Tests**

Sketch an immature mouse ovary and one following ovulation and corpus luteum formation in the mouse.

Sketch a frog spermatozoan.

## EXERCISE 27 – TRANSCRIBING DNA, mRNA AND tRNA TO SEQUENCE A PROTEIN

This exercise is provided to help the operator gain skill in thinking about letter substitutions (representing the purine and pyrimidine bases in nucleic acids). The base sequences presented are not actual ones but rather have been contrived so that when the alphabetical game equivalent is supplied over the amino acid linkages a message is spelled out in English. This, of course, doesn't happen in the biochemistry of cells, but rather the amino acid sequence is relevant and meaningful in terms of cell function without resorting to translations into English.

What is supplied below are the code letters for one DNA strand. Spaces are provided for transcribing the complementary DNA strand and also the mRNA and tRNA. The amino acids associated with these tRNA sequences are given in a table which it may be convenient to cut out and place before you. The game alphabetic equivalent is supplied in the same table.

How would this work out given the DNA strand sequence:

| | CAC | ACG | CAC | CTG | ATC | GTA | CAA | ATA | ACA | GTA |
|---|---|---|---|---|---|---|---|---|---|---|
| Message: | P | E | P | S | I | N | O | G | E | N |
| Amino acids: | His | Thr | His | Leu | Ileu | Val | Glun | Ileu | Thr | Val |
| tRNA Nodocs: | CAC | ACG | CAC | CUG | AUC | GUA | CAA | AUA | ACA | GUA |
| mRNA Codons: | GUG | UGC | GUG | GAC | UAG | CAU | GUU | UAU | UGU | CAU |
| DNA Strand 1: | CAC | ACG | CAC | CTG | ATC | GTA | CAA | ATA | ACA | GTA |
| DNA Strand 2: | GTG | TGC | GTG | GAC | TAG | CAT | GTT | TAT | TGT | CAT |

**Nodocs and Alphabetical Game Equivalents of Amino Acids**

| | | |
|---|---|---|
| AAA | Lys | A |
| AAG | Lys | A |
| AAC | Aspn | B |
| AAU | Aspn | B |
| AGA | Arg | C |
| AGG | Arg | C |
| AGC | Ser | D |
| AGU | Ser | D |
| ACA | Thr | E |
| ACG | Thr | E |
| ACC | Thr | F |
| ACU | Thr | F |
| AUA | Ileu | G |
| AUG | Met | H |
| AUC | Ileu | I |
| AUU | Ileu | I |
| GAA | Glu | J |
| GAG | Glu | J |
| GAC | Asp | K |
| GAU | Asp | K |

| | | |
|---|---|---|
| GCA | Ala | L |
| GCG | Ala | L |
| GCC | Ala | M |
| GCU | Ala | M |
| GUA | Val | N |
| GUG | Val | N |
| GUC | Val | N |
| GUU | Val | N |
| CAA | Glun | O |
| CAG | Glun | O |
| CAC | His | P |
| CAU | His | P |
| CGA | Arg | Q |
| CGG | Arg | Q |
| CGC | Arg | Q |
| CGU | Arg | Q |
| CCA | Pro | R |
| CCG | Pro | R |
| CCC | Pro | R |
| CCU | Pro | R |
| CUA | Leu | S |
| CUG | Leu | S |
| CUC | Leu | T |
| CUU | Leu | T |

| | | |
|---|---|---|
| UAA | Space | |
| UAG | Comma | |
| UAC | Tyr | Period |
| UAU | Tyr | Question |
| UGA | Try | U |
| UGG | Try | U |
| UGC | Cys | V |
| UGU | Cys | V |
| UCA | Ser | W |
| UCG | Ser | W |
| UCC | Ser | X |
| UCU | Ser | X |
| UUA | Leu | Y |
| UUG | Leu | Y |
| UUC | Phe | Z |
| UUU | Phe | Z |

## REPORT SHEETS FOR EXERCISE 27

### Strand One DNA Sequences To Be Decoded

Message:
Amino Acids:
tRNA Nodocs:
mRNA Codons:
DNA Strand 1: GCA AAG GAC ACA TAA ATG AAA CTG CTT ACG
DNA Strand 2:

Message:
Amino Acids:
tRNA Nodocs:
mRNA Codons:
DNA Strand 1: GCA ACA CTT TAA CTC ATG ACG CCC ACG TAA AAT ACA TAA GCA
DNA Strand 2:

Message:
Amino Acids:
tRNA Nodocs:
mRNA Codons:
DNA Strand 1: ATC ACT ACA TAC
DNA Strand 2:

Message:
Amino Acids:
tRNA Nodocs:
mRNA codons:
DNA Strand 1: GCT AAA GAT ACA TAA CTA CAA GCC ACG TAA
DNA Strand 2:

Message:
Amino Acids:
tRNA Nodocs:
mRNA Codons:
DNA Strand 1: CTG AAG CCT CTG AAG CTA CAC AAA CCA ATC GCA GCG AAA TAC
DNA Strand 2:

Message:
Amino Acids:
tRNA Nodocs:
mRNA Codons:
DNA Strand 1: GCG ACA CTC TAA TGA CTG TAA GCT AAA CAT ACA TAA TGA CTA
DNA Strand 2:

Message:
Amino Acids:
tRNA Nodocs:
mRNA Codons:
DNA Strand 1: TAA AAA TAA GCC AAG GTT TAC
DNA Strand 2:

Message:
Amino Acids:
tRNA Nodocs:
mRNA Codons:
DNA Strand 1: TCA ATG AAA CTT TAA ATG AAG CTT ATG TAA ATA CAG AGT TAA TCG CCA CAA
DNA Strand 2:

Message:
Amino Acids:
tRNA Nodocs:
mRNA Codons:
DNA Strand 1: TGG ATA ATG CTT TAT
DNA Strand 2:

Message:
Amino Acids:
tRNA Nodocs:
mRNA Codons:
DNA Strand 1: CTA CTT TGA AGT TTA TAA GTA AAA CTC TGA CCG ACA TAA GTC
DNA Strand 2:

Message:
Amino Acids:
tRNA Nodocs:
mRNA Codons:
DNA Strand 1: CAG CTT TAA AAC CAA CAG GAT CTA TAA CAA GTC GCG TTA
DNA Strand 2:

Message:
Amino Acids:
tRNA Nodocs:
mRNA Codons:
DNA Strand 1: GAC GTT CAA TCG GCG ACG AGC ATA ACA TAA ATT CTG TAA
DNA Strand 2:

Message:
Amino Acids:
tRNA Nodocs:
mRNA Codons:
DNA Strand 1: CAT CAG TCA ACA CCA TAT
DNA Strand 2:

# EXERCISE 28 – DO ENVIRONMENTAL FACTORS AFFECT THE ACTION OF GENES?

(An experiment)

As the procell formed in the primeval oceans, all of its parts were in direct contact with the environment. Then came the invention and application of the cell membrane, which separated more-or-less the inside from the outside. However, we observe evidence which leads us to believe that the conditions under which the living state is maintained are not very much different from what they were in the "beginning." The questions raised are concerned with the independence of gene action from the environment of the cells.

Materials and Equipment (per group of students)

6 Styrofoam drinking cups
Loam, sand, or vermiculite, autoclaved to kill mold spores
Parafilm squares and rubber bands
100 ml. of Gibberellic acid (100 mg./liter)
2 Hand atomizers
20-30 tobacco seeds and 10 seeds each of corn and of beans, all from stocks heterozygous for albinism

Preparations

Punch a hole in the bottom of each of the cups (about ¼ inch). Place a piece of broken cup curved over the hole on the inside to keep soil from spilling out but admitting water. Fill about 3/4 with soil. Cover with Parafilm secured with a rubber band. Place in a tray of water overnight so that the rising water will expel large airspaces from the soil.

Procedure

1. Plant the seeds as follows: Sprinkle the tobacco seeds onto the surface of two pots. Do not cover with soil. Recover with parafilm. Label each pot with your name and tobacco.

2. Use a stick to punch a hole about ½ inch deep in a cup of soil. In fact, punch 5 holes and plant a corn seed in each. Prepare two pots of corn. In a similar way plant two pots of beans. Label with your name and the kind of seed.

3. Place one pot of each kind in a tray of water in a well-lighted place (but not in direct sunlight) along with the pots of other members of the class.

4. Place one pot of each kind in a cabinet or other darkened place in a tray of water (along with similar pots belonging to other members of the class).

5. Allow a week to 10 days for germination. Do not let the trays evaporate to dryness during this time.

6. When the seeds have germinated, count the numbers of green and albino plants for the whole class for those pots grown in the light. What is the appearance of pots grown in the dark?

7. Remove the plants grown in the dark to the light (but not direct sunlight) for several days. What happens? Count the ratio of green and albino plants and compare the ratio with plants grown in the light.

8. Now divide the pots into two groups, each containing half of the plants grown in the light and half of those grown in the dark and later in the light. Measure the height of your plants in centimeters. Designate one group Controls and the other Gibberellic acid.

Spray the control group with water from an atomizer. Spray the Gibberellic acid group with the Gibberellic acid solution, using a separate atomizer. It will be good to do this at some distance from the Control group. Enough solution should be sprayed on to definitely wet the leaves but should not drip off onto the soil.

9. Measure the plants for the first 4 days and again after 7 days, recording your data each time. As plants grow, it may be necessary to place stakes in the pots and tie the plants loosely to them. How do the albino plants get along compared with the normal ones? Determine the mean and standard error for the measurements you make and compare with others to see whether or not their mean ± standard error includes your means. Prepare a graph showing the average height and the standard error for each kind of plant on the ordinate and the time in days since the first spraying on the abscissa (along the bottom).

10. Refer to Exercise 1 to refresh your memory about scientific reports. Write a scientific report on the results of these experiments, interpreting your results and stating what conclusions could be based upon your data.

## REPORT SHEETS FOR EXERCISE 28

**Do Environmental Factors Affect the Action of Genes?**

| PLANT | COLORS | INITIALLY GROWN IN | AFTER DAYS OF GROWTH | DAYS AFTER REMOVAL FROM DARKNESS |
|---|---|---|---|---|
| Tobacco | Green | Light | | |
| | | Dark | | |
| | Albino | Light | | |
| | | Dark | | |
| Beans | Green | Light | | |
| | | Dark | | |
| | Albino | Light | | |
| | | Dark | | |
| Corn | Green | Light | | |
| | | Dark | | |
| | Albino | Light | | |
| | | Dark | | |

| PLANT | TREATMENT | PLANT NO. | COLOR | HEIGHT IN CM. ON DAY | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 1* | 2 | 3 | 4 | 5 |
| Tobacco | Control | 1<br>2<br>3<br>4<br>5 | | | | | | |
| | | Sum<br>Mean<br>S. E. | | | | | | |
| | Gibberellic acid spray | 1<br>2<br>3<br>4<br>5 | | | | | | |
| | | Sum<br>Mean<br>S. E. | | | | | | |

*First day of treatment

**Do Environmental Factors Affect the Action of Genes?**

| PLANT | TREATMENT | PLANT NO. | COLOR | HEIGHT IN CM. ON DAY | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 1* | 2 | 3 | 4 | 7 |
| Beans | Control | 1<br>2<br>3<br>4<br>5 | | | | | | |
| | | Sum<br>Mean<br>S. E. | | | | | | |
| | Gibberellic acid spray | 1<br>2<br>3<br>4<br>5 | | | | | | |
| | | Sum<br>Mean<br>S. E. | | | | | | |
| Corn | Control | 1<br>2<br>3<br>4<br>5 | | | | | | |
| | | Sum<br>Mean<br>S. E. | | | | | | |
| | Gibberellic acid spray | 1<br>2<br>3<br>4<br>5 | | | | | | |
| | | Sum<br>Mean<br>S. E. | | | | | | |

*First day of treatment

# EXERCISE 29 – THE INHERITANCE OF GENETIC TRAITS

## Part A. The Inheritance of Starch and Color in Corn Seeds

The Mendelian principles are known to have wide application among many kinds of organisms. Studying inheritance in corn grains has certain basic advantages. One is the speed with which the observations can be made because the grains are held in place on the ear of corn and this makes counting of individuals easy.

Each corn grain on the ear you will use represents a separate offspring of a cross between the female parent (of which the ear and the corncob are part) and the male parent, which supplied from its tassel the pollen grains that fertilized the ovules which then grew into the corn grains (seeds).

In producing these ears, the plant breeder selected parents that were pure-breeding (homozygous). Two pure strains were crossed and the seeds were then produced belonging to the $F_1$ plants with each other producing the $F_2$ generation. The second kind of cross involved an $F_1$ plant and the recessive parent plant, producing a backcross generation.

Procedure

Work in teams of three students (one counter and two tabulators). Each team will receive an ear of corn. You will not be told whether it is an $F_2$ ear or a backcross ear. This is something you must find out.

**Caution**: Be careful when handling the ears or the kernels will become loose and drop out. **Do not pick the kernels from the ears!**

Examine the ear of corn. Classify the kernels and record phenotypic characters as follows: starchy (smooth), sweet (shrunken), waxy, purple, yellow.

Tabulators should record on a data sheet a column for each type of kernel. To determine number of kernels, the counter should place a pin in the ear at the beginning of the row of kernels where counting starts. He should then call off the phenotypic character of each kernel in each row until he returns to the starting point marked by the pin. As the characters are called out, the tabulators should tally each in the appropriate column on the data sheet.

Total the number of each type of kernel on your ear of corn. Calculate the percentage of each type. Are the percentages close to any ratio of small whole numbers? If so, what? Is your ear of corn an $F_2$ generation or a backcross generation? From your tabulations, can you determine the probable strain of the $F_1$ corn? The probable strain of the parent ears? What trait is dominant? What trait is recessive? Show with Punnett squares the probable cross which produced the progeny represented by your ear of corn. Write a clear statement for each of the Mendelian principles illustrated.

## Part B. Some Problems in Genetics

*Dominance: Mendel's Law*

1. In peas, the gene for tallness (T) is dominant to the gene for shortness (t). What offspring phenotypes would be expected from the following crosses, and in what proportions?

   a. heterozygous (Tt) x heterozygous;

   b. heterozygous x homozygous tall;

   c. homozygous tall x homozygous short.

2. If blue eye color in man is recessive to other colors, could

   a. brown-eyed parents have a blue-eyed child?

   b. blue-eyed parents have a brown-eyed child?

3. Albinism, the inability to synthesize chlorophyll, is a recessive character in a number of plant species. If a tobacco plant known to be heterozygous for albinism is self-pollinated and 600 of its seeds are subsequently germinated,

   a. how many seedlings would be expected to be albino?

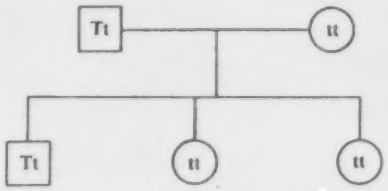
   b. how many would be expected to have the parental genotype?

4. A man and his wife can both taste phenyl thiourea. They have four children, two of whom are unable to taste. What are the parental genotypes?

*The 1:2:1 Phenotypic Ratio*

5. Construct pedigree diagrams for the following families, giving the possible genotypes for each member. Represent males with a square and females with a circle, and write the genotype alongside or inside the symbol.

Example: A man who is a taster has a non-taster wife and they have a taster son and two non-taster daughters,




   a. A man and his wife are both tasters and they have two taster sons, a taster daughter, and a non-taster daughter.

   b. A taster man has a taster son, a non-taster son, and a taster daughter by his first marriage and two non-taster daughters by his second marriage. His first wife was never tested but his second wife was a non-taster.

   c. The daughter of his first marriage subsequently married a non-taster and produced eight children, all of whom were tasters.

*Genes Affecting Visibility*

6. A farmer crossed his roan bull with three groups of cows. Cows of Group A were white; cows of Group B were red; and cows of Group C were roan.

   What proportions of the different coat colors would he expect in the offspring of each group of cows?

7. If the same farmer decided to make his Shorthorn herd exclusively roan coat by selling all white and red calves would the color of bull he used make any difference, economically?

8. When Poggy, a colored male hamster, was mated with Zoe, a white female, the offspring were all colored. When Zoe was later mated with Prince, who was colored, some of this second litter were white.

   a. What might you conclude about the genotypes of Poggy, Prince, and Zoe with respect to coat color?

b. If an animal of the first litter were to be mated with a colored animal of the second litter, what offspring phenotypic ratios might be expected?

c. What offspring might be expected from a cross between an animal of the first litter and a white animal from the second litter?

*Observed and Expected Ratios*

9. In an investigation of the nature of length of leg in the domestic fowl the following two crosses were made and the numbers of offspring recorded:

| | NUMBER OF SHORT OFFSPRING | NUMBER OF NORMAL OFFSPRING |
|---|---|---|
| 1. normal x short leg | 48 | 51 |
| 2. short leg x short leg | 55 | 28 |

From these figures what might be concluded about the action of this gene?

10. Brachyphalangy in man is a condition in which the middle bone of the fingers is greatly shortened and usually fused to the next bone, with the result that the fingers appear to have only two joints. A brachyphalangic person is heterozygous for a lethal gene; the homozygous offspring is born without digits and with gross skeletal defects which soon cause death.

a. In a marriage between a brachyphalangic man and a normal woman what proportion of their children would be expected to be brachyphalangic?

b. Show this diagrammatically.

c. What ratio of brachyphalangic to normal children would be expected in a family in which both parents are brachyphalangic?

d. Show this diagrammatically.

*Phenyotypic Ratios Where Both Alleles Show Dominance: The 9:3:3:1 Ratio*

11. If a maize plant heterozygous for the alleles for pigmy and crinkly-leaf (both recessive to normal size of plant and normal leaf) is self-pollinated and 160 of the seeds are subsequently collected and germinated, how many would you expect to show

a. crinkly leaves?

b. normal size?

c. normal leaves and normal size?

d. normal leaves and pigmy?

*$F_2$ Genotypic Ratios*

12. In a certain breed of domestic fowl, pea comb is dominant to single comb, but feather color shows absence of dominance; black feathers and white feathers are homozygous, e.g., BB and bb respectively, whereas the heterozygote Bb gives "blue" feather.

From crosses between birds heterozygous for both alleles, what proportion of the offspring would be expected to be

a. pea combed?

b. black feathered?

c. blue feathered?

d. pea combed and blue feathered?

e. single combed and white?

13. In *Drosophila*, curved wing (c) and forked bristle (f) are recessive to the normal or wild type wing and bristle shape (expressed as + in each case). Show diagrammatically, the following crosses:

a. +c +f x cc ff;

b. ++ +f x +c ff;

c. +c +f x +c ff.

14. What proportion of the offspring from the cross in question 13c would be expected to show the wild-type phenotypes for both alleles?

*The Punnett Square*

15. In the fowl two pairs of alleles (Rr and Pp) regulate the shape of comb. Two cocks, each of which has a pea-shaped comb, are mated to five hens as follows:

| | Hen | Comb | Offspring comb ratios |
|---|---|---|---|
| | (A | walnut | 3 walnut: 3 pea: 1 rose: 1 single |
| Cock X x | (B | pea | 3 pea: 1 single |
| | (C | rose | 1 walnut: 1 pea: 1 rose: 1 single |
| | )D | single | all pea |
| Cock Y x | )E | rose | all walnut |

What simple genetic explanation accounts for the inheritance of comb shape and what are the genotypes of the cocks and hens above?

16. In peas the gene for round seed (R) is dominant to wrinkled (r), and yellow seed coat (Y) is dominant to green (y). Consider a cross between peas heterozygous for both alleles;

a. what proportion of the seeds produced would be expected to be wrinkled?

b. what proportion would be expected to be yellow?

c. what proportion would be expected to contain a homozygous pair of alleles?

*Significance: The Chi-Square Test*

17. In man a recessive gene for a severe disease of the blood vessels is heterozygous in about 0.05% of the population. A man whose brother died from the disease is contemplating marriage and consults a genetics counselor because he is worried about the chances of a child of his having the disease. What advice might the counselor give?

18. If the man in question 17 marries his first cousin what are his chances of having a child with the disease?

19. What is the probability that a family of four children will consist of

a. four boys?

b. three boys and a girl?

c. two boys and two girls?

d. one boy and three girls?

e. four girls?

What is the connection between this question and the previous one?

20. When pink sweet peas were self-pollinated and the seeds were collected and sown, the following flower colors were obtained:

red 36; pink 56; white 28.

Are these results consistent with the hypothesis that pink flowers are heterozygous for a single pair of color alleles, showing absence of dominance?

*Sex Chromosomes*

21. If a sex-linked gene is a recessive lethal, causing death and reabsorption of the embryo at an early stage,

a. what proportion of the offspring would be expected to be females?

b. and what proportion of the offspring would be expected to be carriers of the gene from a mating between two mammals, the female of which carries the gene in its heterozygous state?

22. In cats, a coat color gene is located on the non-homologus portion of the X chromosome. BB or B– is black, bb is yellow, and Bb is tortoiseshell. Are these statements true or false; give the reasons.

(i) A mating of a black tom with a yellow tabby could give female tortoiseshell kittens.

(ii) The same mating can give male tortoiseshell kittens.

(iii) A yellow male mated with a tortoiseshell female could give black male kittens.

(iv) The same mating could give black female kittens.

(v) A tortoiseshell tabby can have tortoiseshell kittens whatever color tomcat she mates with.

23. A normal sighted woman whose father was red-green colorblind married a man with normal vision. What ratios of genotypes and phenotypes would be expected among their children?

*Sex Linkage*

24. The gene for the ability to taste PTU is autosomal and dominant to non-tasting. Red/green colorblindness is sex-linked and recessive.

A man and his wife, both of whom can taste PTU and have normal vision, have a child who is colorblind and a non-taster.

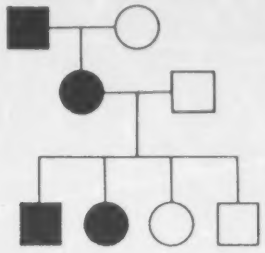
a. What is the child's sex?

b. What is the probability (expected occurrence) of this child's phenotype in their children?

c. What is the probability that a child will be a taster with normal vision, like its parents?

d. What is the probability that a child will be a non-taster and colorblind, unlike its parents?

25. Consider the following pedigree covering three generations of a mammal. The symbol ● depicts a female showing a certain trait in the phenotype and □ represents a normal male.



a. Does this show evidence of sex-linkage?

b. Under what conditions could this pedigree represent the inheritance of a sex-linked gene?

c. Can a father and his son both be haemophiliacs?

*Skin Color In Man*

26. On the basis of Davenport's findings that skin color in man depends on the pigment genes present on two unlinked loci (two pairs of genes), and that white skin can be represented as aaaa or $a^4$, how would you describe the

a. black skin genotype?

b. mulatto?

c. dark mulatto?

d. light mulatto?

27. Show, algebraically, the cross between

a. two dark-mulatto people;

b. two light mulattos;

c. one mulatto and one dark mulatto.

For each, show the algebraic expression of their possible offspring genotypes.

28. a. What proportion of the children of a marriage between two light mulatto parents would be expected to have white skins?

b. What proportion would be expected to have the same colored skins as their parents?

29. Three alleles for the C locus (coat color) in the rabbit are c (albino), which is recessive to $c^{ch}$ (chinchilla coat), which in turn is recessive to C (the allele for full coat color).

In a randomly mating population of rabbits the following gene frequencies were determined:

C 0.2;
$c^{ch}$ 0.6;
c 0.2.

What proportions of coat color (expressed as percentages) would you expect to find in that population?

30. The tyrannical overlord of the planet Sirius III decided to improve the breed of his subjects and issued a Eugenical Edict whereby all individuals having more than the customary three digits were to be sterilized before they reached sexual maturity. This edict was made when it was found that the frequency of polydactyly in the population was one in sixteen.

Like all the inhabitants so far discovered in the galaxy these aliens showed a normal Mendelian pattern of inheritance, and polydactyly was single-gene controlled and recessive.

a. Complete the following table to show the frequency of polydactylous individuals in each generation:

| NUMBER OF GENERATIONS | 0 | 2 | 4 | 6 | 8 | 10 |
|---|---|---|---|---|---|---|
| frequency of polydactyly | 0.0625 | | | | | |

b. Plot the frequency of polydactyly against the number of generations on a graph.

c. If the time of one generation of the inhabitants of Sirius III is 25 years, how long will it take to reduce the incidence of polydactyly to the level of one in 10,000?

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These problems are from Harrison, David (1970), *Problems in Genetics*, Reading, Mass. Addison-Wesley Publishing Company.

## REPORT SHEETS FOR EXERCISE 29

### Part A. Inheritance of Starch and Color in Corn Seeds

| CHARACTERS | TALLY | TOTALS |
|---|---|---|
| Starchy (smooth) | | |
| Sweet (wrinkled) | | |
| Waxy | | |
| Yellow | | |
| Purple | | |
| | | |

Ratio of starch to sweet is _____ : _____

Ratio of purple to white _____ : _____

Is your ear of corn in the $F_1$ or $F_2$ generation? _____

Which of the above traits are dominant and which recessive?

Form a Punnit square for the possible genotypes producing the phenotypes observed.

| | | | |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |

What Mendelian principles are illustrated?

# EXERCISE 30 – SEARCHING FOR BACTERIA RESISTANT TO STREPTOMYCIN AND INCREASED SALT CONCENTRATIONS[1]

An hypothesis for this exercise could be the question, "Do genetic recombinations occurring during meiosis result in offsprings that are better able to meet adversities in the environment?" We shall use an antibiotic, streptomycin, and dehydration resulting from "unphysiologically" high concentrations of sodium chloride as aids for the exploration of this question.

Materials and Equipment

A pure culture of E. coli in broth (24 hours)
3 Nutrient agar petri plates
2 each Nutrient agar petri plates containing .85%, 1.5%, 3% and 6% NaCl
3 1-ml. sterile (disposable) pipettes
1 glass spreader made from a Pasteur pipette
1 bunsen burner
1 inoculating loop
1 small beaker
1 hand atomizer
.05% streptomycin
95% alcohol
Wax pencil

## Part A. Searching for Bacteria Resistant to Streptomycin

Procedure

1. Use asceptic techniques throughout. See Exercise 5.

2. Mark the covers of two nutrient agar plates with your initials and E. coli.

**Partner A.** Flame the culture tube and withdraw .5 ml. of culture with a sterile 1 ml. pipette. Raise one side of the petri dish covers and pipette .25 ml. onto the surface of each dish.

**Partner B.** While the transfer of organisms is being made, dip the sealed and bent Pasteur pipette into alcohol and flame. Let cool. Spread the inoculum evenly over the surface of both plates.

3. Allow the plates to stand until the liquid is all absorbed by the agar, then invert the plates. They are then incubated at 22-28°C (room temperature) for 6 hours to overnight. After this initial incubation period you probably will not see the bacterial colonies due to their minute size. Sterilize a glass spreader by flaming and letting it cool. Lift one side of one of the dishes and gently respread the surface. Label this dish "Spread." Label the other one "Control."

4. Use the atomizer to spray a fine and gentle mist over the agar surface of both plates. Be careful not to apply so much of the solution that it runs. Let the plates sit upright until liquid is absorbed, then invert.

5. Incubate the plates at 22-28°C for 24 to 48 hours. Count the colonies present on each plate. Record your data.

[1] Part A courtesy Martin J. Carey, Clark College, Atlanta, Georgia.

6. You will no doubt ask yourself whether or not it was possible that a drop of penicillin mist may not have landed on some bacteria and these are the ones that grew into colonies. To test that hypothesis, use a flamed inoculating loop and transfer some organisms from an apparently resistant colony to a fresh tube of broth. Respread on a nutrient agar plate and spray with .05% streptomycin. What alternative methods could be used?

**Part B. Resistance to Increased Salt Concentration**

All enzymes require water in order to work. If water is not available then cell metabolism will stop. This kind of intercellular drought can be brought about by exosmosis, that is by subjecting the cells to a hypertonic environmental medium. Can some cells overcome this situation? If so, how do you think that they might reasonably do it?

Procedure

Use asceptic technique throughout. See Exercise 5.

1. Obtain 5 petri plates containing 0, .85%, 1.5%, 3% and 6% NaCl in the agar medium. Mark them accordingly.

2. Flame the tube of 24 hour culture of E. Coli and transfer .25 ml. to each plate (draw up at least 1.25 ml.). **Partner B**–Dip a bent glass spreader into alcohol. Flame and cool. Spread the inoculum evenly over their surface of the plates, starting with the least salt concentration and working up. (Why would it not be advisable to start at the highest concentration and work toward the least concentrated?)

3. Incubate at 22-28°C (room temperature) for 48 hours. At the end of that time rate the amount of growth on the plates as follows:

+++, the amount of growth on the control (0% NaCl) plate
++, somewhat less growth than on the control plate
+, a trace of growth, a few colonies
0, no growth at all.

4. Transfer organisms from the colonies formed to fresh broth of the corresponding salt concentration. Rate the amount of growth after 48 hours by the same plus-system used above.

## REPORT SHEET FOR EXERCISE 30

**Part A. Searching for Bacteria Resistant to Streptomycin**

| TREATMENT | COLONIES |
|---|---|
| Control | _____ |
| Spread | _____ |

What result from reculture of an apparently resistant colony?

**Part B. Resistance to Increased Salt Concentration**

Amount of Growth after 48 Hours at _____°C. in These Per Cents of NaCl

| | | Growth on Reculture |
|---|---|---|
| 0 | _____ | _____ |
| .85 | _____ | _____ |
| 1.5 | _____ | _____ |
| 3.0 | _____ | _____ |
| 6.0 | _____ | _____ |

Questions

1. If more colonies appear on the spread plates than on the unspread ones, what explanation can one give to support that result?

2. Did the streptomycin-resistant organisms "breed true"?

3. By what mechanism can the bacteria survive the higher concentrations of NaCl?

## EXERCISE 31 – THE INHERITANCE OF SPECIFIC PROTEINS

Bloodletting was a medical practice that extended from antiquity into the 20th Century. The medicinal leech (Hirudo medicinalis) could be purchased in drug stores in the United States up into the 1920's or thereabouts. At the turn of the century, some physicians began to wonder if bleeding the sick might be a cause of death for many of them. They reasoned that it might be helpful to give a patient blood instead of taking it away from him.

The first of these tissue transplants (blood being a connective tissue) was successful but those that followed were not. Through the pioneering work of Moss, Jansky and Landsteiner, working independently, it was discovered that something in blood plasma was causing some blood donations to clump (or agglutinate). They didn't know what was causing this effect so they called it an "antibody," a term coined by Louis Pasteur less than 50 years previously.

We have learned a great deal since 1903. We know that most of the antigens are proteins and that the antibodies are proteins of the gamma globulin group of blood plasma proteins. The protein that is called A or the one that is called B will be present in many kinds of cells, maybe all of the cells, of the body. Some people form both proteins A and B and others don't form either one. Antibodies react most strongly with specific proteins and as the structure of proteins begins to vary from that, the antibody becomes less able to act upon it. Since we can stimulate the production of antibody proteins in a number of animals–rabbits, horses, etc.–by injecting them with the antigen protein we wish to identify, it becomes a very convenient tool for locating specific proteins without the longer processes of physical-chemical analysis.

Man forms a few antibodies spontaneously. One is against Pneumococcus type 7, but the more common is production of antibodies against the kind of cell membrane protein (A or B) which is not made by that individual. The antibody protein against protein A is called anti-A or alpha. The one against protein B is called anti-B or beta. People who form protein A spontaneously form anti-B or beta antibody. Those that form protein B spontaneously form anti-A or alpha antibody. That was what caused Drs. Moss, Jansky and Landsteiner trouble back in 1903.

About 85% of the white population in the United States, and a slightly higher percent among Negroes and orientals, form another group of membrane proteins. These are designated C, D and E, which constitute the Rhesus monkey factor (Rh factor). (Proteins A and B are also found among other Primates.) Antibodies against the Rh factor are not formed spontaneously but are formed if Rh protein is infused into the blood of an Rh-negative person, that is one that does not form Rh protein.

A considerable list of proteins in cell membranes, including red blood corpuscle membranes, have now been identified. Their names–Kell, Duffy, Lewis, etc.–denote the names of the patients in which these proteins were first discovered to be missing.

Proteins A, B, C, D, and E are quite different. However, there are some substitutions of a single amino acid into the hemoglobin chains which may have far reaching effects and are responsible for such blood disorders as thallasemia and sickle-cell anemia. The resulting change is not easily detected with antibodies as specific as they are. Sicke cell anemia (a homozygous condition) and sickle-cell trait (the heterozygous condition) can be detected by a simple test.

The genes $I^A$ and $I^B$ which regulate the production of proteins A and B are not dominant to each other. So, a person who has both genes $I^A$ and $I^B$ will produce both proteins A and B and therefore be of type AB. A person who is homozygously recessive for these genes ($I^a$ and $I^b$) will

form neither protein A nor B and is designated as type 0. Genes $I^A$ and $I^B$ are dominant to their recessives of course, so that a person of type A can be either homozygous ($I^AI^A$) or heterozygous ($I^AI^a$) or more properly

| | |
|---|---|
| Type A | $I^AI^AI^bI^b$ or $I^AI^aI^bI^b$ |
| Type B | $I^aI^aI^BI^B$ or $I^aI^aI^BI^b$ |
| Type AB | $I^AI^AI^BI^B$ or $I^AI^aI^BI^B$ or $I^AI^AI^BI^b$ or $I^AI^aI^BI^b$ |
| Type O | $I^aI^aI^bI^b$ |

There are about 3 billion combinations for the known blood cell proteins, indicating that each person on earth probably has a protein "fingerprint" discernable if all the known proteins were typed for that blood.

Procedure

Preparations

1. Dry a clean slide from alcohol using a lint-free cloth or paper towelling. Draw two circles about ¼ inch in diameter with a wax pencil. Label the left circle A and the right one B. Put your initials on the end of the slide.

2. Place about 1 ml (1 inch) of normal (0.89%) NaCl in a small (7 mm.) test tube and place the tube in a rack or other support.

3. Wash your hands if you are the subject. Wipe a fingertip on the left hand (if you are right-handed) with some 70% alcohol on a pledget of cotton.

Wipe the fingertip next with ether on a pledget of cotton. The evaporation of the ether helps to rapidly remove the alcohol (which will destroy red blood corpuscles), and at the same time the cooling effect draws blood into the fingertip.

Obtaining A Few Drops of Fresh Blood

4. Have your partner open a packet containing a sterile, disposable, metal lancet, viz:

5. Place the left hand palm-upward on the table with the cleaned fingertip extended. Have your partner plunge the lancet firmly into the ball of the fingertip. The skin at this point is about ¼ inch thick so the lancet should go all the way to the stop. Remove and discard the lancet.

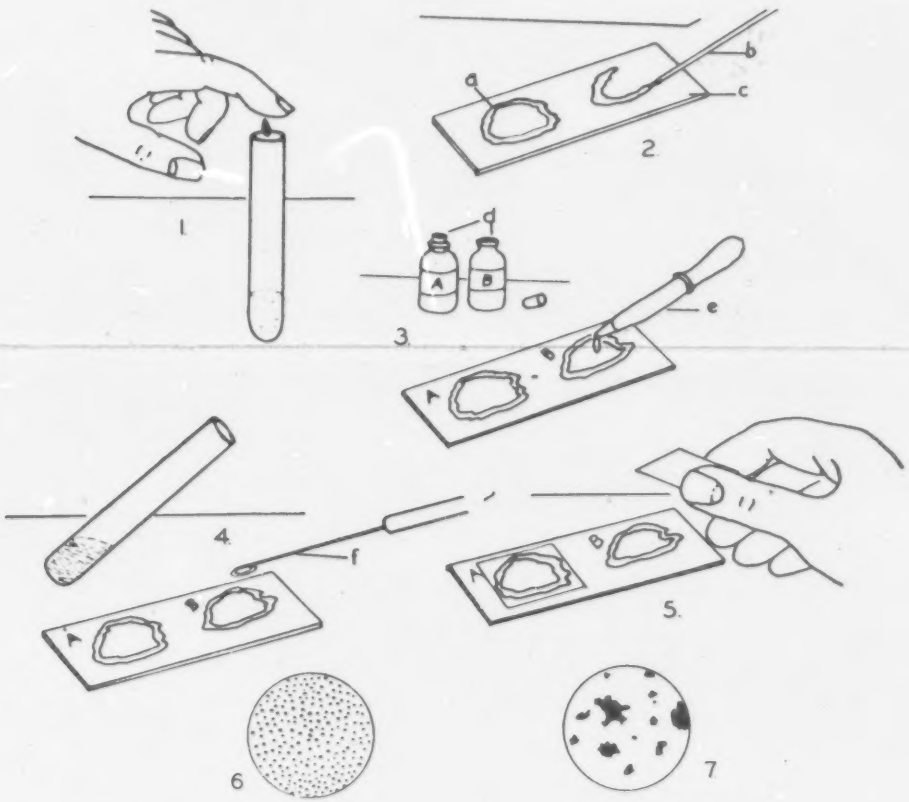
6. Two or three drops of blood can be encouraged to flow out by gentle pressure on the fingertip. Add this blood to the tube, shaking to mix. Make a medium pink suspension. If it is red, add more saline. How will this prevent pseudoagglutination?

ABO Typing Using Anti-A and Anti-B Serum

7. Take the slide with marked circles to the teacher's table and obtain a drop of Anti-A serum in the A circle and a drop of Anti-B serum in the B circle. Return to your work place with the slide and add a drop of the blood suspension prepared in Step 6. Be careful not to let the dropper touch either of the drops of serum. Mix each circle by stirring with *different ends* of a toothpick or applicator stick.

8. Examine the preparations under the low power (10X) objective of the microscope. Type A blood will clump on the A side and Type B blood will clump on the B side. Type O will not clump in either circle, but Type AB will clump in both.

An Alternate Procedure[1]




**Open Slide Blood Typing Method.** (Illustrating the procedure that may be followed in blood typing).
1–collecting two or three drops of blood in a test tube with a few cc. of normal saline;
2–by means of a vaseline "gun," b, a circle of vaseline is placed on each end of the slide, C;
3–the one circle is labeled A and the other B and a drop of A and B type sera, d, is added to each respectively by means of a small pipette, e;
4–a small drop of diluted blood of unknown type in the test tube is added to each drop with a platinum wire loop (flame well between drops);
5–coverslips are placed over each to prevent evaporation. The vaseline acts as a seal.
6–unagglutinated blood and
7–agglutinated blood as it appears under the microscope.

[1]From Pace, McCashland and Riedesel, 1964, *Laboratory Manual for Vertebrate Physiology*, Minneapolis, Burgess Publishing Co., p. 158.

c. A Problem in Cross-Matching

| ABO TYPE | CELL ANTIGEN | PLASMA OR SERUM ANTIBODY |
|---|---|---|
| O | Neither | alpha and beta |
| A | A | beta |
| B | B | alpha |
| AB | A and B | Neither |

Type A or Type B blood can be used to determine any other type if the titer of antibody is high enough and there are no inhibitors (H in Type O blood). Let us use Type A blood as an example and assume no interference.

**Procedure for Obtaining Materials.** Separate the cells from plasma by centrifugation or from serum by letting blood samples clot. One sample here is Type A blood. Now mix Type A cells with serum or plasma of the untyped blood and mix Type A serum (containing Anti-B or beta antibody) with cells of the untyped blood. Mix and let stand. Look for agglutination.

Work out this problem using Type B blood cells and serum to type unknown types.

| CASE | BLOOD TYPE | TYPE A CELLS AND UNTYPED SERUM | TYPE A SERUM (BETA) AND UNTYPED CELLS |
|---|---|---|---|
| I | B | Clumped B, Ø | Clumped B, AB |
| II | A | Not Clumped A, AB | Not Clumped A, Ø |
| III | O | Clumped B, O | Not Clumped A, O |
| IV | AB | Not Clumped A, AB | Clumped B, AB |

NOTE: Where the Untyped Cells are in Type A Serum (beta), one must write down the ABO Type of the blood with (or without) the antibody concerned.

d. Rh Types (Types C, c, D, d, E, e)

The Rh factors are cell proteins C, D, and E. If one is positive for any of these, the capital letters are used to show its presence as the result of genetic dominance for the formation of these specific proteins. Small letters are used to indicate the absence of these proteins due to genetic recessiveness (Rh Negativity).

(1) Dry a clean slide from alcohol using paper towelling. Handle the slide by its edges.

(2) Draw three ¾-inch diameter circles, label them C, D, and E. Write your initials on the end of the slide and place it on the Rh-typing box for a few minutes to warm up.

(3) Obtain from the teacher a drop of Anti-C, Anti-D and Anti-E sera in the proper circles. Add a drop of *undiluted* blood to each. Mix with separate toothpicks and place on the Rh-typing box. Shake for 4 minutes, observing results at 1 minute intervals.

(4) Report your results for C, D, and E separately, using capital letters for positive reactions, and small letters for negative ones.

# FREQUENCY OF Rh TYPES AND GENOTYPES IN WHITE POPULATION

| Rh TYPE | FREQUENCY (Percent) | GENOTYPE | SYMBOL | FREQUENCY (Percent) | SERUM REACTIONS C | D | E | c | e |
|---|---|---|---|---|---|---|---|---|---|
| cde | 15.1 | cde/cde | rr | 15.1020 | O | O | O | + | + |
| Cde | .8 | Cde/Cde | $R'R'$ | .0097 | + | O | O | O | + |
| | | Cde/cdeq | $R'r$ | .7644 | + | O | O | + | + |
| cDe | 2.1 | cDe/cDe | $R_0R_0$ | .0659 | O | + | O | + | + |
| | | cDe/cde | $R_0r$ | 1.9950 | O | + | O | + | + |
| cdE | .9 | cdE/cdE | $R''R''$ | .0141 | O | O | + | + | O |
| | | cdE/cde | $R''r$ | .9235 | O | O | + | + | + |
| CDe | 53.4 | CDe/CDe | $R_1R_1$ | 17.6803 | + | + | O | O | + |
| | | CDe/Cde | $R_1R'$ | .8270 | + | + | O | O | + |
| | | CDe/cDe | $R_1R_0$ | 2.1585 | + | + | O | + | + |
| | | CDe/cde | $R_1r$ | 32.6808 | + | + | O | + | + |
| | | Cde/cDe | $R'R_0$ | .0505 | + | + | O | + | + |
| cDE | 14.1 | cDE/cDE | $R_2R_2$ | 1.9906 | O | + | + | + | O |
| | | cDE/cDe | $R_2R_0$ | .7243 | O | + | + | + | + |
| | | cDE/cdE | $R_2R''$ | .3353 | O | + | + | + | O |
| | | CdE/cde | $R_2r$ | 10.9657 | O | + | + | + | + |
| | | cde/cdE | $R_0R''$ | .0610 | O | + | + | + | + |
| CdE | .03 | CdE/CdE | $R_yR_y$ | .0000 | + | O | + | O | O |
| | | CdE/Cde | $R_yR'$ | .0001 | + | O | + | O | + |
| | | CdE/cdE | $R_yR''$ | .0001 | + | O | + | + | O |
| | | CdE/cde | $R_yr$ | .0039 | + | O | + | + | + |
| | | Cde/cdE | $R'R''$ | .0234 | + | O | + | + | + |
| CDE | 13.6 | CDE/CDE | $R_zR_z$ | .0006 | + | + | + | O | O |
| | | CDE/CDe | $R_zR_1$ | .2048 | + | + | + | O | + |
| | | CDE/cDE | $R_zR_2$ | .0687 | + | + | + | + | O |
| | | CDE/CdE | $R_zR_y$ | .0000 | + | + | + | O | O |
| | | CDE/Cde | $R_zR'$ | .0048 | + | + | + | O | + |
| | | CDe/CdE | $R_1R_y$ | .0042 | + | + | + | O | + |
| | | CDE/cDe | $R_zR_0$ | .0125 | + | + | + | + | + |
| | | CDe/cDE | $R_1R_2$ | 11.8648 | + | + | + | + | + |
| | | CDE/cdE | $R_zR''$ | .0058 | + | + | + | + | O |
| | | cDE/CdE | $R_2R_y$ | .0014 | + | + | + | + | O |
| | | CDE/cde | $R_zr$ | .1893 | + | + | + | + | + |
| | | CDe/cdE | $R_1R''$ | .9992 | + | + | + | + | + |
| | | cDE/Cde | $R_2R'$ | .2775 | + | + | + | + | + |
| | | CdE/cDe | $R_yR_0$ | .0003 | + | + | + | + | + |

**Part B. Test for Sickling**

Mix a drop of blood and a drop of 20% sodium sulfite solution on a microscope slide. Cover with a coverslip and seal with melted petrolatum (petroleum jelly) applied with a small brush. Let stand. Sickle cell disease will cause sickling in about 15 minutes. Sickle cell trait will take about 30 minutes or a little longer.

## REPORT SHEET FOR EXERCISE 31

**Reactions to Antisera:**

Anti-A _____ Anti-B _____ Blood Phenotype _____

Anti-C _____ Anti-D _____ Anti-E _____ Phenotype _____

Possible Genotype _____

The sickling test was positive in _____ minutes negative (circle one)

| CASE | UNKNOWN SERUM AND TYPE B CELLS | UNKNOWN TYPE CELLS AND SERUM FROM TYPE B (ANTI-A OR BETA) | TYPE |
|---|---|---|---|
| I | Clumped | Clumped | |
| II | Not Clumped | Not Clumped | |
| III | Clumped | Not Clumped | |
| IV | Not Clumped | Clumped | |

# EXERCISE 32 – PHOTOSYNTHESIS AND THE SYNTHESIS OF STARCH

Material and Equipment

Hot plate
2 150-250 ml. beakers
Scissors
250 watt lamps on stands
Vacuum pump or aspirator
2 Test tubes with 1-hole stoppers, glass and rubber tubing
2 Hammond clamps
Geranium plant in the light and plant left in the dark 48 or more hours
95% ethanol
Lugol's iodine
Dilute pyrogallol solution
Elodea sprigs

## Part A. Effect of Light on Photosynthetic Activity

Preparations

Enough geranium plants have been obtained so that there are at least two leaves for each working group in the laboratory. These have been divided into two equal groups and Group 1 placed in a dark cabinet for at least 48 hours before class meets. Group 2 plants have remained in the light.

Take two strips of paper about .5 x 6 inches and write your name, section, and No. 1 on one and No. 2 on the other.

Procedure

1. Select a leaf on a plant in Group 1 and place your No. 1 identification strip as a loose bracelet around the petiole, fastening the ends together with a staple. Do the same for a leaf in Group 2 using your No. 2 identification strip.

2. Using sharp scissors or a razor blade, cut off the distal half of your leaf No. 1 and the right half of your leaf No. 2. The veining patterns will identify these for a while.

3. Place the leaves in a beaker and cover with water. Boil for about 2 minutes to extract water-soluble pigments.

4. Transfer the leaves to a beaker containing 95% ethanol. Boil on a hot plate away from flames until all of the green pigment has been extracted. Remove the leaves and blot them dry between paper towels.

5. Place the leaf halves in a half petri dish and cover with Lugol's iodine solution. Rinse away the excess iodine with gently running tap water.

6. Dry the leaves between blotting paper or towelling in a leaf press for use in your report later.

7. The Group 1 plants will now be left in the light and the Group 2 plants will be placed in a dark cabinet for at least 48 hours or until next laboratory period.

8. At the next laboratory period remove the other halves of the leaves from the plants and repeat Steps 3 through 6.

9. Divide the leaves tested among the members of the group and attach them with rubber cement to the spaces provided in the Report Sheet.

**Part B. Is Oxygen A Product of Photosynthesis?**

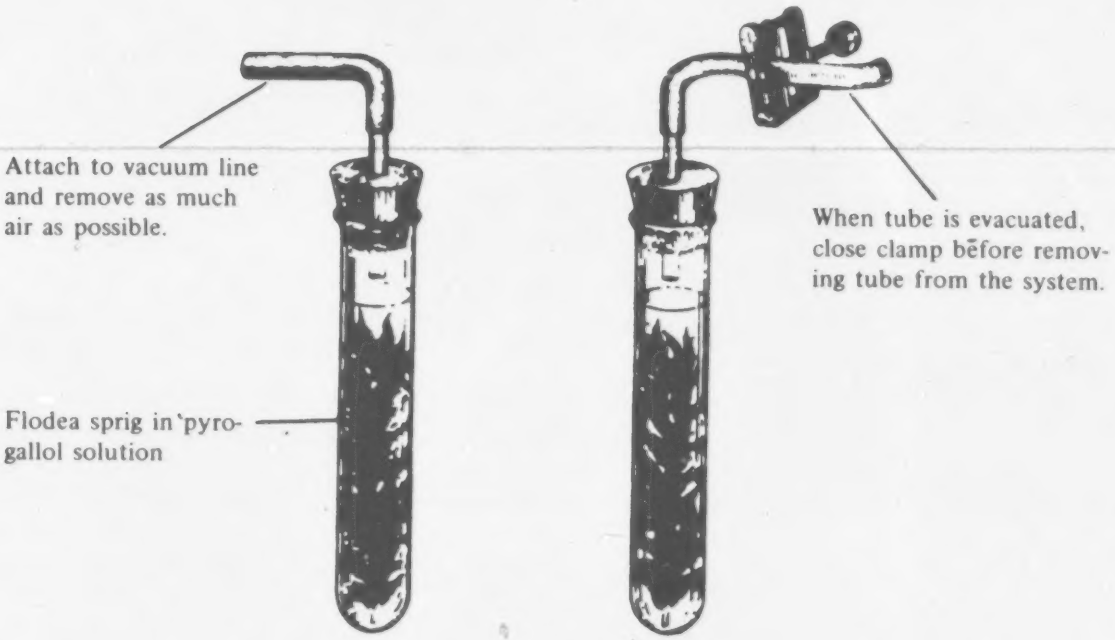
Procedure

1. Fill two test tubes about ¾ full of a freshly prepared solution of pyrogallol. The teacher will provide a demonstration of what happens to pyrogallol when oxygen (from the breath for example) is bubbled through the solution.

2. Insert a fresh sprig of Elodea (water weed) into each test tube. Insert a rubber stopper equipped with a glass tube, short segment of rubber tubing, and a Hammond clamp.

3. Attach the tubes to a vacuum pump or to a water aspirator (with a water trap interposed before the tube) and remove as much air (oxygen) as possible. Close the clamp before removal from the vacuum system.

4. Place one tube in bright light (sunlight or a 250 watt bulb). Examine periodically and compare with the other tube which has been placed in a dark cabinet.


Attach to vacuum line and remove as much air as possible.
Flodea sprig in pyro-gallol solution
When tube is evacuated, close clamp before removing tube from the system.

**Part C. IN VITRO Synthesis of Starch**

Materials and Equipment

| | |
|---|---|
| Irish potatoes | 0.01 M NaCN or NaF (both are poison) |
| Knife | Lugol's solution |
| Food chopper | 0.01 M Glucose-1-Phosphate |
| Beaker | Centrifuge tubes |
| Suction flask with Buchner funnel | 8 Test tubes and test tube rack |
| Vacuum line (through water trap) | Disposable spot trays |
| Centrifuge | 0.01 M Glucose (Dextrose) |
| Filter paper | .2% Starch solution |
| Wax pencil | .2 M Potassium acid phosphate $KH_2PO_4$ |

Crude Preparation Containing Phosphorylase

1. Wash about 100 grams of potatoes and cut into small pieces with a knife. Grind these in the food chopper, catching the juice (which may turn brown).

2. To the chopped potato and its juices add 40 ml. of .01 M sodium cyanide or of sodium fluoride, to inhibit other enzymes, particularly phosphatases.

3. Filter the homogenate through a double layer of cheesecloth. This filtrate must have the starch removed. This can be done by filtering by suction through a double filter paper on a Buchner funnel or by centrifugation for 3 to 5 minutes. Test the final solution for the presence of starch by transferring a little to a spot plate or test tube and adding a few drops of Lugol's iodine solution.

4. While preparing the enzyme, start a boiling water bath. Place 10 ml. of the enzyme preparation in a test tube and boil about 10 minutes to inactivate the enzyme.

Procedure

1. Number test tubes 1 through 7 with wax pencil. Make these preparations:

Tube 1: 3 ml. .01 M glucose, 1 drop .2% starch solution
Tube 2: 3 ml. .01 M glucose-1-phosphate, 1 drop starch
Tube 3: 3 ml. .01 M glucose-1-phosphate
Tube 4: 3 ml. .01 M glucose-1-phosphate, 1 drop starch
Tube 5: 3 ml. .01 M glucose-1-phosphate, 1 ml. .2M $KH_2PO_4$, 1 drop .2% starch
Tube 6: 3 ml. .2% starch, 1 ml. .2 M $KH_2PO_4$
Tube 7: 3 ml. .2% starch solution, 1 ml. .2 M $KH_2PO_4$

Add a few drops of Lugol's iodine solution to 7 depressions on a spot tray.

2. Note the time then add 3 ml. of the enzyme preparation to Tubes 1, 2, 3, 5, and 6. Add 3 ml. of the *boiled* enzyme preparation to Tubes 4 and 7. Shake each tube to mix well. Remove a few drops of mixture from each tube and drop in the appropriately numbered depression on the spot tray. *Use a separate Pasteur pipette for each transfer.* Return the pipette to the proper mixture for later use.

3. Incubate the mixtures for at least 30 minutes at room temperature.

4. At the end of that time repeat the Lugol's iodine test for starch on all of the mixtures and record your results.

## REPORT SHEET FOR EXERCISE 32

**Part A. Effect of Light on Photosynthetic Activity**

Results of iodine tests on leaf fragments extracted with water and ethanol.

| | |
|---|---|
| Piece of Leaf No. 1 from plant after _____ days in the dark. | Piece of same Leaf No. 1 after _____ days in the light. |
| Piece of Leaf No. 2 kept in the light. | Piece of same Leaf No. 2 after _____ days in the dark. |

Explain the above results.

**Part B. Is Oxygen A Product of Photosynthesis?**

1. What effect does oxygen have on the color of the pyrogallol solution?

2. What happens to the pyrogallol in the tube brightly illuminated as compared with the tube kept in the dark?

**Part C. IN VITRO Synthesis of Starch**

| TUBE | CONTENTS | | COLOR OF IODINE TEST AT THE START | COLOR OF IODINE TEST AFTER | MINUTES |
|---|---|---|---|---|---|
| 1 | Glucose + starch + enzyme | 1 | | | |
| 2 | G-1-P + starch + enzyme | 2 | | | |
| 3 | G-1-P + enzyme | 3 | | | |
| 4 | G-1-P + boiled enzyme | 4 | | | |
| 5 | G-1-P + $PO_4$ + starch + enzyme | 5 | | | |
| 6 | $PO_4$ + starch + enzyme | 6 | | | |
| 7 | $PO_4$ + starch + boiled enzyme | 7 | | | |

What information does each tube yield in this experiment?

**Part C. IN VITRO Synthesis of Starch** (continued)

What is the role (or roles) of the potassium phosphate?

Consult your text and find answers to these questions:

What are the final products of the reactions driven by light?

What does carbon dioxide combine with in the carbon dioxide fixation reaction?

How many molecules of triose sugar (glyceraldehyde) are formed as the result of fixing 6 molec. of $CO_2$?

Glucose is a product of the reactions leading from an excess of glyceraldehyde. Yet, unlike in animals, glucose is not the sugar that is transported from the site of photosynthesis to the site of food storage (usually as starch). What sugar is transported in plants?

What is the ultimate source of the energy in glucose?

# EXERCISE 33 – DIGESTION OF FOODS

Materials and Equipment

Test tubes, 15 x 150 mm (15)
Spot plate or disposable tray
Test tube bath
Bunsen burner
Thermometer
Beaker, 1000 ml
Ice
Paraffin
Hard-boiled egg white
Cream (10 ml)
Acetic acid, dilute (30 ml)
Starch paste (60 ml)
Lugol's solution (30 ml) diluted 10:1
Benedict's solution (60 ml)
Litmus solution (10 ml)
Pancreatin solution, 5% (30 ml)
Pepsin solution, 5% (30 ml)
Sodium bicarbonate solution, 1% (30 ml)
Hydrochloric acid, 0.1N (30 ml)
Hydrochloric acid solution, 0.5% (30 ml)
Sodium hydroxide solution, 0.5% (30 ml)
Chloroform (10 ml)
Pasteur pipettes

**Part A. Salivary Digestion**

1. Obtain some 1% malt diastase made up in Ringer's solution or in buffer at pH 7.4, and containing .9% NaCl as an activator, or

Chew a piece of clean paraffin for a few minutes and collect the saliva produced in a clean beaker. If your mouth feels dry, suck upon a piece of fresh lemon to stimulate saliva flow. Dilute the saliva with an equal amount of water and filter through cotton.

2. Test the pH of the saliva or diastase with pHydrion paper. Record your results. Boil 5 ml. of the enzyme (diastase or saliva) and let cool for use in Step 4.

3. To a small portion of saliva add a few drops of 1% acetic acid. A precipitate will indicate the presence of mucin (a glycoprotein lubricant).

4. Mark five test tubes with wax pencil S1, S2, S3, S4 and S5. To all tubes add 5 ml. of 0.5% starch solution. To tube S2 add 5 ml. of the saliva solution. To tube S3 add 5 ml. of the saliva solution and place immediately in a beaker of ice. To tube S4 add 5 ml. of the saliva solution that has been boiled and cooled. To tube S5 add 5 ml. of the saliva solution and 1 ml. .1N HCl.

5. Obtain a disposable spot tray. Mark 5 columns S1 through S5 with wax pencil.

6. Insert a clean Pasteur pipette into each test tube. At three minute intervals transfer a few drops of the incubating digests to an appropriate depression in the spot tray. Add a drop of diluted Lugol's solution to the sample.

7. When the iodine is no longer changed by the digest in tube S2, transfer about 2 ml. of each tube to a clean set of marked test tubes.

Add 2 ml. of Benedict's reagent to each tube and boil for three minutes. Record the colors.

**Part B. Gastric Digestion**

1. A small piece of hard boiled egg white, or preferably some purified fibrin is placed in each of 5 test tubes labeled G1, G2, G3, G4 and G5.

2. To tubes G1, G2, G4 and G5 add 5 ml. of a 5% solution of commercial pepsin
   To tubes G2 and G3 add 5 ml. of water
   To tubes G1, G3 and G4 add 5 ml. of .5% HCl
   To tube G5 add 5 ml. of .5% NaOH.

3. Place the tubes in a water bath at 37-40°C and observe every several (20) minutes to watch the progress of the hydrolysis.

4. Record your results.

**Part C. Pancreatic Digestion of Egg White**

1. Label three test tubes P1, P2, P3. Add 5 ml. of a 5% commercial pancreatin solution to each tube.

2. To tube P1 add a 5mm. cube of hard boiled egg and 5 ml. of 1% sodium bicarbonate
   To tube P2 add a 5 mm. cube of hard boiled egg and 5 ml. of water
   To tube P3 add a 5 mm. cube of hard boiled egg and 5 ml. of .5% HCl.

Place in a water bath at 37-40°C and observe every few minutes to follow the progress of the hydrolysis.

**Part D. Pancreatic Digestion of Fat**

1. Add about 3 ml. of cream to a test tube. Add twice the volume of 5% pancreatin and a few drops of bromthymol blue. Then add dropwise just enough .1N NaOH to turn the mixture blue.

2. Place in a water bath at 37-40°C and observe at intervals for two hours.

## REPORT SHEET FOR EXERCISE 33

### Part A. Salivary Digestion

| MINUTES AFTER START | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| Color of Benedict's Test after _____ Min. | | | | | |

### Part B. Gastric Digestion

| DIGESTION TIME | G1 | G2 | G3 | G4 | G5 |
|---|---|---|---|---|---|
| | | | | | |

### Part C. Pancreatic Digestion of Egg White

| TIME | PER CENT DIGESTED | | |
|---|---|---|---|
| | P1 | P2 | P3 |
| | | | |

### Part D.

Color at Start: _____

Color after _____ minutes: _____

**Part A. Salivary Digestion**

What effect did the following have on the rate of starch digestion as compared with Tube S2? Explain.

Tube S3, placed in ice.

Tube S4, containing boiled enzyme.

Tube S5, in acid pH.

**Part B. Gastric Digestion**

Was HCl and water effective in hydrolyzing the protein? Why?

Why was there digestion at one pH and not at the other?

**Part C. Pancreatic Digestion**

What was the role of sodium bicarbonate in tube P1?

What effect did HCl have on the digestion rate?

**Part D. Pancreatic Digestion of Fat**

Why did the bromthymol blue indicator change color?

| PART OF ALIMENTARY TRACT | GLAND(S) | NAME OF ENZYME OR MATERIAL | CLASS OF FOOD ACTED UPON | PRODUCTS OF DIGESTION |
|---|---|---|---|---|
| | Parotid, Submaxillary Sublingual | | | |
| Stomach | | | | |
| Small Intestine | Liver | Bile | | |
| | Pancreas | | Fats | |
| | | Trypsinogen | | |
| | | Chymotrypsinogen | | |
| | | Carboxypeptidase | | |
| | Crypts of Lieberkuhn | | Maltose | |
| | | | Sucrose | |
| | | | | |

Write a report on how the secretion of pancreatin is regulated from the small intestine.

# EXERCISE 34 – MEASUREMENT OF OXYGEN USE

## Part A. Assembling the Apparatus

Materials and Equipment

A plastic pan 12 in. x 18 in. approximately
2 Support stands
2 Ring supports
2 quart or other size bottles with wide mouths and equal volumes
2 2-hole rubber stoppers to fit the mouths of the jar
2 10-ml pipettes
2 feet of ¼-inch (outside diameter) glass tubing
Rubber tubing (¼-inch inside diameter)
File
Bunsen burner
2 Pinch-type clamps

Procedure

1. Place the plastic pan on the bases of the support stands.

2. Place the bottles in the pan and bring the ring supports in position to hold the jars securely against the bottom of the pan (to keep them from floating later when water will be added to the pan.*

3. Cut off part of the tip of the pipettes to enlarge the hole enough to permit the rapid passage of water. However, don't cut so much that it will be difficult to get a piece of rubber tubing over the end.

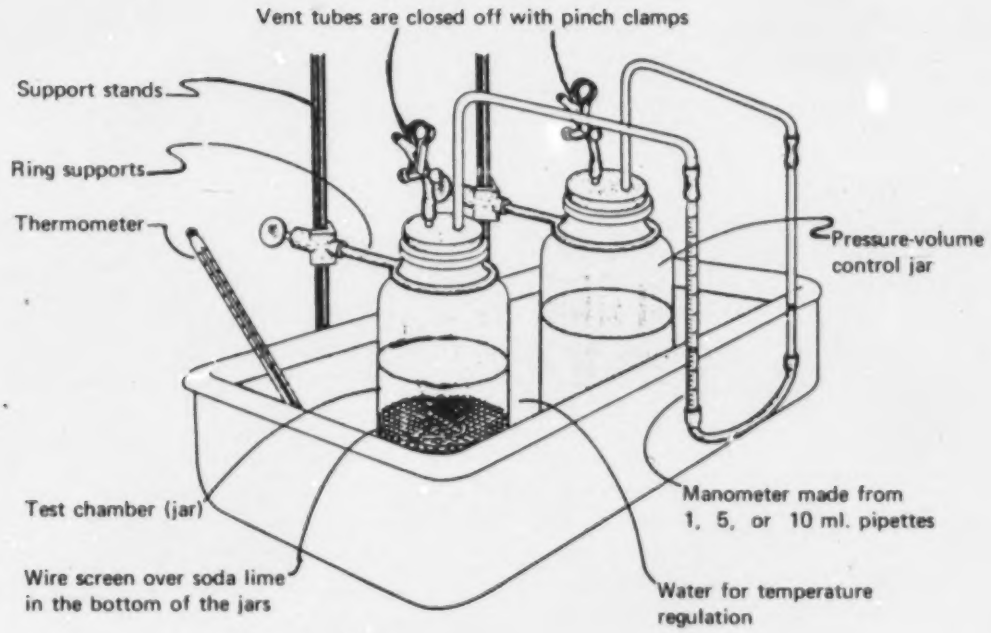
4. Insert a short and a longer piece of glass into the two-holed stoppers, being sure to lubricate the stopper and the glass either with water or glycerine.

5. Bend the tubing as shown in the illustration.

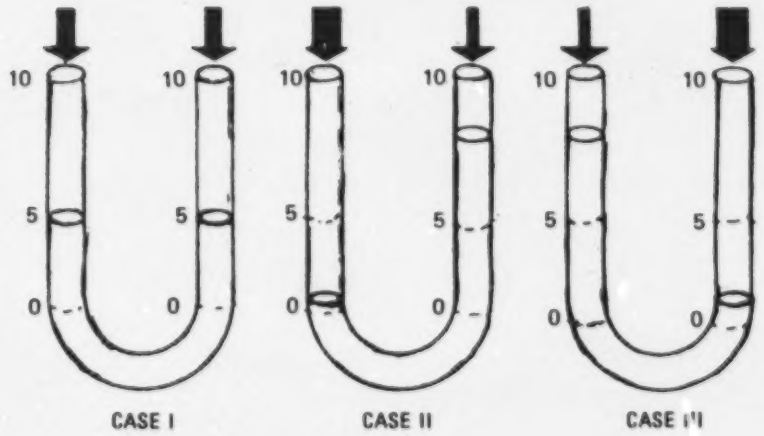
6. Attach the pipettes to the longer tubes coming from the stoppers with short pieces of rubber tubing and add a piece of rubber tubing to the shorter piece of glass. Put a pinch-type clamp on the latter.

7. Disconnect the connection to the top of one of the pipettes and add water (which may be colored with a dye for easier reading) making use of a Pasteur pipette. Fill until the pipette is half-full when held in position. When the filling is complete, reconnect to the rest of the apparatus. The manometers may also be filled by injecting the fluid into the bottom connecting tube, making use of a syringe and a fine (25 gauge) needle.

*At this point about .5 inch of soda lime can be placed in each jar and covered with wire gauze, or a bag of soda lime can be suspended from the top using adhesive tape around an inside protrusion of the glass tubing.


Vent tubes are closed off with pinch clamps
Support stands
Ring supports
Thermometer
Pressure-volume control jar
Test chamber (jar)
Manometer made from 1, 5, or 10 ml. pipettes
Wire screen over soda lime in the bottom of the jars
Water for temperature regulation

**Apparatus For Making Measurements of Oxygen Use**




In *Case I* both jars have the same gas pressure and therefore the same amount of gas. In *Case II*, if gas pressure is generated in the test jar the water level will be depressed. If the right side were open to the air it might rise higher, but the closed jar keeps the pressure on that side increased the same as on the left. In *Case III* gases are being taken up in the test chamber, creating a partial vacuum and drawing the water upward. The fall of the water on the right side creates an equal partial vacuum on that side. The pipettes are calibrated in ml. and so give the volume of gas used directly. For more refined work the volume would be reduced to standard conditions of 1 atmosphere (760 mm. Hg.) at Zero degrees C.

**Part B. Measuring Oxygen Use**

This apparatus can be used to measure the oxygen use of a variety of living things, the main requirement being that there be enough material to get a measurement within a reasonable time (laboratory periods being what they are). Try one or more of these combinations:

Dry peas, then germinating peas. (Insert an aluminum foil liner in the test jar to keep the soda lime dry.)

A mouse at room temperature, at a warmer-than-room temperature and in an ice-water bath.

Yeast cells in media with and without sugar. (In this case the soda lime will have to be suspended from the top of the jar.)

Oxygen use or production by plants under strong illumination and in darkness. (Soda lime suspended from the top of the jar.)

Repeat each test three times. Average the results and find the standard error.

Procedure

1. Place the material or organism to be tested in the test chamber. In the case of animals cover the jar with a wire screen so that the animal may have plenty of air while he calms down. Open the vents on both jars. When the animal is calm put the stoppers in place and let the organisms come to temperature equilibration for about 5 minutes. Then, note the time the level of the meniscus of the water on the test manometer, and then close the vents on both jars.

2. Since in this case you will not be able to measure the use of more than 5 ml. of oxygen at a time, it may take only a short time for an active animal to use up that much. Mark the time it takes to use 5 ml. of oxygen or mark the amount of oxygen used after a given amount of time, say 30 minutes in the case of something with a low oxygen-uptake rate. When the fluid reaches the top of the scale, stop time and open the test chamber vent.

Examples:

A mouse at 28°C used up 5 ml. oxygen in 150 seconds. The mouse weighed 25 grams. Determine its use of oxygen per gram per hour.

$$\frac{\text{ml. } O_2 \text{ used}}{\text{time in min.}} \times \frac{60 \text{ min.}}{1 \text{ hr.}} = \frac{5}{2.5} \times \frac{60}{1} = 120 \text{ ml. } O_2/\text{hour}$$

ml./hr. ÷ body wt. = 120/25 = 4.8 ml. $O_2$/gram body wt./hour

One hundred grams of soaked peas used up 3.0 ml. in 30 minutes. Determine its oxygen use per gram per hour.

$$\frac{\text{ml. } O_2 \text{ used}}{\text{time}} \times \frac{60 \text{ min.}}{1 \text{ hr.}} = \frac{3.0}{30} \times \frac{60}{1} = \frac{180}{30} = 6 \text{ ml./hour}$$

ml. per hr./wt. = 6/100 = .06 ml. $O_2$/gram/hour

## REPORT SHEET FOR EXERCISE 34

**Measurement of Oxygen Use**

| WHAT WAS TESTED? | WT. IN GRAMS | TEMP. °C. | VOL. $O_2$ USED | TIME IN MIN. | Ml. $O_2$ PER HR | Ml. $O_2$/GRAM/ HOUR |
|---|---|---|---|---|---|---|
| | | | | | | |
| Sum<br>Mean<br>± S.E. | | | | | | |
| | | | | | | |
| Sum<br>Mean<br>± S.E. | | | | | | |
| | | | | | | |
| Sum<br>Mean<br>± S.E. | | | | | | |
| | | | | | | |

## EXERCISE 35 – URINALYSIS (URINE ANALYSIS)

Urinalysis serves an ever important role as an aid in the diagnosis and prognosis of diseases. However, like other laboratory tests, these analyses are supplementary to other findings by the physician. Since urine constituents are derived from plasma by glomerular filtration or by tubular secretion, such procedures as outlined below give some indication of disorders in kidney function as well as indications of plasma concentrations. A particular disease may be characterized by (1) the presence in the urine of some substance not normally present or (2) a greatly increased or decreased amount of some normal constituent. Both the quantity and the nature of the food eaten may have a marked influence on the composition of the urine; these must be known to evaluate properly the results of urinalyses. Collect a morning sample of about 100 ml.

Materials and Equipment (per student)

Test tube rack
Test tubes
pH indicator paper
Bunsen burner
Acetic acid
Benedict's solution
Microscope slides
Cover glasses
Microscope
Concentrated nitric acid
Clinitest brand tablets
Albumin
Saturated picric acid
10% NaOH
Watchglass
Saturated sodium bicarbonate
Dilute nitric acid
Dilute HCl
Dilute barium chloride solution in dropping bottle
Acetest brand acetone-testing tablets
Concentrated hydrochloric acid
3% Sulfosalicylic acid
5% Copper sulfate
Chloroform
"Tes-Tape" for urine sugar
Clinical centrifuge
Dilute silver nitrate solution in dropping bottle
Disposable urine cup with cover and a few drops of toluene
Central laboratory supply station with centrifuge and pathological urine

Procedure

**Physical Examination**

*Color:* Colorless, very pale, pale, amber (normal), or dark.

*Odor:* Odorless, aromatic (due to urinod), putrid, ammoniacal, fecal, or due to the added preservative.

*Transparency:* Record as clear or cloudy. If cloudy, indicate if with or without a sediment or macroscopic shreds.

*pH:* Use a pHydrion paper. Tear off a piece of tape about 1½ inches in length. Add a drop of urine and compare with the color chart on the container. Other methods may be used.

Specific Gravity and Total Solids in Urine.

The specific gravity of the urine is proportional to the concentration of solutes. The grams of solute per liter of urine may be calculated roughly by multiplying the second and third decimal figures of the specific gravity by 2.6, Long's coefficient. This is, of course, an approximation for substances differ widely in their effect on specific gravity. The specific gravity of a liter of urine is raised 0.001 by 3.6 grams of urea, by 1.47 grams of NaCl, by 3.8 grams of sodium acid phosphate, by 2.7 grams of glucose, or by 3.9 grams of albumin. This coefficient is valid for urine at 25°C. Each 3°C change from 25°C will lower specific gravity .001 below, or raise it .001 above 25°C.

*Procedure:* Use an urinometer. Pour enough urine into the glass cylinder to float the indicator (hydrometer). Make sure that the indicator is not touching the side or bottom of the cylinder. Read the number of the specific gravity scale at the meniscus of the urine. The numbers on the urinometer scale represent the second and third decimal places of the specific gravity, that is, 20 is recorded as 1.020. Record the temperature of the specimen.

Calculate the total solids in the twenty-four hour excretion of 1500 ml. of urine. The second and third decimal figures from the specific gravity reading multiplied by Long's coefficient (2.6) will give the grams of solids per liter of urine.

**Chemical Examination**

*pH:* See above.

Tests for Proteins in Urine

*Principle.* Because it is difficult for large molecules to pass through the membrane of the capillary walls, normal urine contains only very small amounts of protein (less than 75 mg./day). This will consist of serum albumin, a variety of enzymes (pepsin, trypsin, lipase, amylase) and mucin derived from the lower urinary tract. The following tests are designed to detect abnormal amounts of protein in urine but not to react with the small amount normally present.

*The Nitric Acid Test.* Transfer about ½ inch of concentrated nitric acid to a test tube. Holding the tube in a slanted position slowly and carefully add about ½ inch of urine in such a way that it floats on the acid. Mix the layers slightly to give zones with different proportions of urine and acid. One of these zones will have the proper conditions for precipitating any protein present. Albumin will form a white layer.

*The Sulfosalicylic Acid Test.* (Albumin or globulin) To 1 ml. of urine in a test tube add 2 ml. of 3% sulfosalicylic acid. A turbidity or precipitate indicates the presence of albumin or globulin. Precipitation may be increased by warming the tube.

*Acetic Acid Test.* To 5 ml. of urine in a test tube, add one ml. of 50% acetic acid and 3 ml. of saturated NaCl. Heat the mixture gradually to boiling. If albumin or globulin is present, a white precipitate will appear. The presence of bile acids or urates may yield a precipitate after the addition of solution which goes back into solution upon heating, indicates the presence of the "Bence-Jones protein" which is found in certain pathologic conditions.

If you are in doubt as to whether a positive test was obtained, repeat the test using a control mixture in which water is substituted for the urine.

*Coagulation or Boiling Test.* Fill a test tube about ¾ full with urine of acid pH. Place in boiling water bath (preferred) or incline the tube slightly and gently heat the upper layer to boiling in the flame of a burner, holding the tube at the bottom. Rotate the tube gradually as the upper layers become heated. If the urine remains clear after standing for several minutes, no albumin is present. If an opalescence, a white cloud, or a turbidity forms in the heated portions, it may be due to the presence of (1) albumin or (2) salts. Add a few drops of 1% acetic acid to the heated portion and boil again. If the cloud clears up entirely without producing an effervescence, the cloud was due to phosphates. If effervescence was produced when the acid was added, the cloud was due to carbonates. If the turbidity persists (or becomes more intensified after adding the acid and heating) it is due to albumin.

*Acetone:* Place an acetest tablet in a syracuse watch glass. Add a drop of urine. If acetone and/or diacetic acid are present, a purple color will develop which can be compared with the color scale provided. The color develops because of a reaction with sodium nitroprusside under alkaline conditions, the color being proportional to the amount of nitroprusside complexed.

*Indican (Indoxyl Sulfuric Acid)*

**Principle**: Trypotophan is degraded by intestinal bacteria to indole, indoxyl, skatol and skatoxyl, which contribute to the foul odor of feces. Some indoxyl is absorbed and conjugated by the liver with sulfate yield indican, the potassium salt of indoxyl sulfuric acid, which is excreted in the urine. Increased urinary indican is found in constipation and lower intestinal obstruction. In this test HC1 splits off the sulfate and the indoxyl condenses and is oxidized to indigo blue.

**Procedure**: Place 5 ml. of urine in a test tube and add 0.5 ml. 5% copper sulfate. In the hood add 2.5 ml. chloroform (keep away from flames) and 6 ml. of concentrated HC1. Mix by inverting the tube several times (close with rubber stopper). The appearance of a blue color in the chloroform layer indicates the presence of indican. The intensity of the blue color is roughly quantitative of the amount of indican present.

*Sugar Tests*

Benedict's Test. To 5 ml. of Benedicts qualitative reagent in a test tube add 8 drops of urine. Mix thoroughly and boil in a boiling water bath for 2 minutes. If sugar is absent the solution will remain blue. If sugar is present, increasing quantities will form a precipitate that will be opalescent green, yellow, orange, orange-brown, or bright red in color.

*The Clinitest Tablet Test for Glucose*

Place 10 drops of urine and 10 drops of water in a test tube. Add one Clinitest tablet for glucose determination (blue and white in color). This is essentially the ingredients in Benedict's solution. Wait 15 seconds after boiling ceases and then mix the contents well with shaking, A negative test is blue in color. If there is brown, yellow, orange or red color, compare with the color chart at the table in the front of the room. Record the mg% present. Place 2 drops of urine on a piece of "Tes-Tape" 1½ inches long. Compare color after 1 minute with color scale.

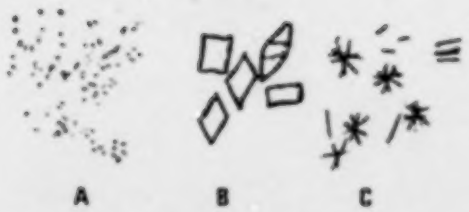
When you have completed the Chemical Tests, clean up your glass ware and check your material back in. Sponge off the top of your work space, then go on to the microscopic examination.

**Microscopic Examination**

Put exactly 10 ml. of urine in a test tube of the designated size and place your initials in wax pencil on the same. Put it in the centrifuge. When it is properly loaded the laboratory teacher will spin it at 2500 rpm for 4 minutes. This low speed is to avoid damaging the elements of the sediment.

To remove the sediment, decant as much of the urine back into the collection cup, as possible. Prepare a hanging drop in a depression slide and observe under the microscope with low and high power.

## Unorganized and Chemical Sediments



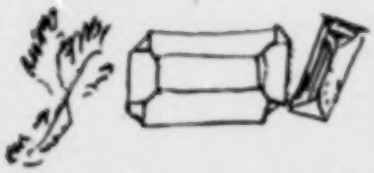

1. Amorphous deposits. A uric acid in acid urine, phosphate in alkaline urine.
A. Uric phosphate; B. Uric acid; C. Calcium phosphate

2. Tyrosine crystals

3. Leucine
A. Pure
B. Impure

3. Crystals of cystine

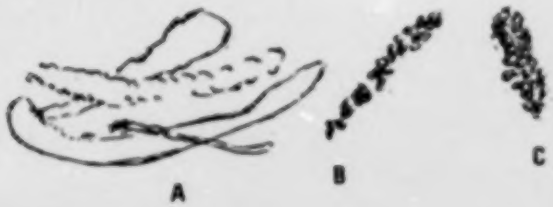
4. Crystalline phosphates



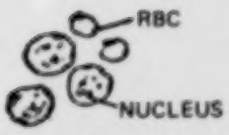
5. Triple phosphates

## Organized Sediments

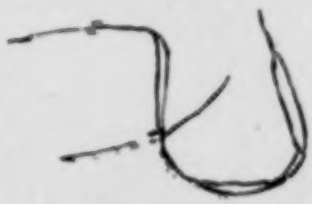
1. Urinary epithelial cells.
A. Surface cornified type
B. Deeper types




2. Types of Casts. A. Hyaline, colloid or waxy, depending on appearance. B. A leucocyte cast. C. Form of blood, fatty and granular casts, depending upon the content.




3. Pus cells are leucocytes. They are about twice as big as red blood corpuscles.



4. Mucoid cylindroids

5. Spermatozoa

## REPORT SHEET FOR EXERCISE 35

**Urinalysis**

*Physical Examination*

Color:
Odor:
Specific Gravity:
24 Hour solids in 1500 ml.:
Transparency:
Sediment:
Time of day collected:
Type of preservative added:

*Chemical Examination*

pH:
Nitric acid test for protein:
Sulfosalicylic acid test for albumin or globulin:
Acetic acid test: Albumin or globulin?
Bile acids or urates?
"Bence-Jones protein"?
Coagulation or Boiling Test for albumin:
Acetone by Acetest tablet test):
Indican (Indoxyl sulfuric acid):
Sugar (Benedict's Test):
Sugar (Tes-Tape Test):
Sugar (Clinitest Tablet Test):

*Microscopic Examination* (Indicate whether present or absent and how much if present)

Amorphous sediment:
Bacteria:
Molds and Yeasts:
Casts:
Crystals:
Cylindroids:
Erythrocytes (RBC):
Leukocytes and pus cells:
Epithelial cells:
Mucus:
Spermatozoa:

Time of last meal:
What did you eat?:

## EXERCISE 36 – REFLEX ACTIONS

Sterile thread
Sterile rubber-tipped depressor stick
Neurological hammer

**Part A. Cranial Reflexes** (Those Mediated Through the Brain)

1. Corneal Reflex. Touch the cornea of your partner's eye gently with a piece of sterile thread. Use both a frontal and a lateral approach. Is there any difference? How does such a reflex help the individual?

2. Light Reflex. Have your partner cover one eye with his cupped hand. After a few seconds have him move it away quickly. What happens to the pupil of that eye? The light is transduced at the retina into nerve impulses which leave the eye by way of the second cranial nerve, the optic nerve. What cranial nerves control dilation and constriction of the pupil?

3. Consenual Reflex. Cover the right eye and note the dilation of the pupil in the left one. Now have your partner uncover his right eye quickly. What happens to the pupil of the left eye?

4. Accommodation Reflex. Watch the pupil of your partner's eye while he focuses on a near object (a few inches away) and then on a distant one (20 or more feet away). Since this deals with the sharpness of the visual image on the retina as interpreted at some comparator with experience on what is sharp focus, diagram a possible reflex network for controlling accommodation.

5. Pharyngeal Reflex. Touch the back of your partner's pharynx with a sterile probe (a piece of clean soft rubber tubing on a narrow depressor stick is fine because biting it will not result in injury). Have your partner warm up the probe by holding it in his mouth for a minute or so. Now touch the back of the pharynx. How does he react?

**Part B. Spinal Reflexes** (Those Mediated Primarily Through the Spinal Cord)

Patellar Reflex. This is also known as the knee-jerk reflex and is typical of postural reflexes mediated through the cord and influenced by impulses from the cerebellum and other brain centers.

Have your partner sit on the edge of a table so that the legs hang free. Strike the patellar tendon just below the patella (knee cap) with the edge of the hand or with a neurological hammer. Repeat several times to be sure the response is not voluntary. Try it with the lower leg partly extended. If the response seems slight, try this maneuver: have the subject hook the fingers of one hand inside the hooked fingers of the other hand and then pull hard, creating strong isometric contractions of the arm muscles while the patellar tendon is struck. What effect does this maneuver have on response?

Now change places with your partner so that he can observe the reflexes in you. Then answer the questions on the Report Sheet.

## REPORT SHEET FOR EXERCISE 36

### Part A. Cranial Reflexes

1. Describe the corneal reflex.

2. What was the difference between the frontal and lateral approaches?

3. How does the corneal reflex serve the body?

4. Describe the light reflex.

5. What cranial nerve causes dilation of the pupil?

   Which cranial nerve causes contraction of the pupil?

6. Is the light reflex present during sleep?

7. What is meant by a mydriatic drug? Name one.

8. What is meant by a meiotic drug? Name one.

9. Give two reasons why size of the pupil is helpful in interpreting visual images of our environment.

10. Diagram a possible reflex network for accommodation.

11. Describe the pharyngeal reflex. What was the effect of warming the probe?

**Part B. Spinal Reflex**

12. Describe the patellar reflex.

13 What effect did the pulling maneuver have on the reflex? Give a good reason why this is so.

14. What is the relationship between the knee jerk reflex and tabes dorsalis?

15. Diagram a spinal reflex arc indicating the receptor, sensory neuron, dorsal root ganglion, cross section of spinal cord·motor neuron, ventral root of motor neuron, and effector.

## EXERCISE 37 – INFLUENCE OF THYROID HORMONE ON RATE OF DEVELOPMENT

*(Demonstration or Special Project for Two or More Students)*

Materials and Equipment

7 Aquaria or one-gallon jars
7 Rocks or bricks to form easily-accessible island for froglets
35 or more tadpoles (just before or in the hind limb-bud stage)
Supplies of pond water
Stock solutions of the following made up 20 mg. in 200 ml. pond water:
- Thyroxine or Triiodotyrosine
- Thiouracil
- Lugol's iodine solution (1%) diluted 1:100 = 20 mg./200 ml. in pond water

Procedure

Label the aquaria from 1 to 7
Add a liter of pond water. Then add to
No. 1–10 μg. Thyroxine (or Triiodotyrosine as directed by the instructor)
No. 2–10 micrograms thyroxine (10 ml. of stock/liter pond water)
No. 3–Pond water control
No. 4–20 micrograms iodine (20 ml. diluted Lugol's/liter pond water)
No. 5–10 micrograms thiouracil
No. 6–20 micrograms thiouracil
No. 7–20 micrograms thiouracil + 20 micrograms thyroxine

Replace the aquarium solutions every two or three days or as needed.

Things to be checked:

Is the mouth small with a beak-like upper lip or wide, reaching back under the eyes?
Are the intestines coiled neatly or have they extended and uncoiled?
Are the hind limbs present?
The forelimbs are formed beneath the skin then break through an opening. Which one exteriorized first, the right or left?
What is the size of the tail?
Have the ear drums appeared yet?

Observe animals every two or three days and enter date under feature in the answer sheet when that feature appears.

Remove dead animals immediately but do not replace them from stock!

## REPORT SHEET FOR EXERCISE 37

| MONTH/DAY THAT FEATURES ARE PRESENT | | | | | | FORELIMB FREE | |
|---|---|---|---|---|---|---|---|
| AQUARIUM NO. | SUCKING MOUTH | BIG MOUTH | COILED INTESTINE | UNCOILED INTESTINE | HIND LIMBS | RIGHT | LEFT |
| #1 | | | | | | | |
| #2 | | | | | | | |
| #3 | | | | | | | |
| #4 | | | | | | | |
| #5 | | | | | | | |
| #6 | | | | | | | |
| #7 | | | | | | | |

| | TAIL LENGTH | | | | | |
|---|---|---|---|---|---|---|
| AQUARIUM NO. | OVER 20 MM. | 20-10 MM. | 10-5 MM. | UNDER 5 MM. | EAR DRUMS | |
| #1 | | | | | | |
| #2 | | | | | | |
| #3 | | | | | | |
| #4 | | | | | | |
| #5 | | | | | | |
| #6 | | | | | | |
| #7 | | | | | | |

1. Look up the formula for thyroxine (or triiodotyrosine if you used it).
Resource: a textbook of Endocrinology.

2. What is the formula for theouracil?

3. What effect was produced by thyroxine? Were these effects proportional to the dosage used?

4. What effects were produced by thiouracil?

5. What would be a good reason for having included treatment with iodine alone, knowing that iodine is a constituent of the thyroid hormone?

6. How could you check to determine if the changes obtained with thyroxine (or triiodotyrosine) are reversible?

# EXERCISE 38 – A STUDY OF PLANT COMMUNITIES

*(A Field Exercise)*

If you have completed Exercise 17 and Exercises 20 through 23, you have a speaking acquaintance with the variety of living things and with some of the ways in which they reproduce. That is, your information includes something about fruits, seeds, fruiting bodies and other reproductive structures to be seen in the field. Exercises 6, 28 through 30 and 32 dealt in part with such environmental factors as light, temperature and salinity on the behavior of cells and organisms. Therefore, this exercise and the one that follows may come at the end of the course where it will have the effect of helping to integrate a great many facts about nature, or it may come early in the course in an effort to help you see some rather generalized biological problems facing our society and scientists. Scientists as a group, see themselves trying to solve such problems.

In Biology the word community has the same meaning it has in sociology, that is, it is conceptualized as several different kinds or conditions of the same species and of different species interacting together in such a way as to produce the phenomenon of an increasing or decreasing body of many parts. If we say that an organism is the association of organ systems, morphologically integrated and physiologically coordinated into an individual, then a superorganism may be described as an association of individuals, morphologically integrated and physiologically coordinated to do things that the individual organism cannot do alone. In a way Gestalt concepts come into play so that the whole ends up being more than just the sum of its parts, it is an organization that fits together in a peculiar way in order to be the vital superorganism that is a community.

Materials and Equipment

Field notebook and pencil
Tape measure at least 33 feet long
Photographic exposure meter

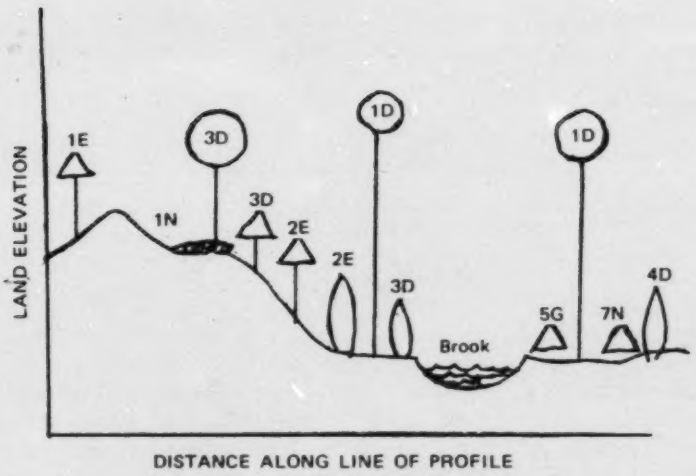
Procedures

**Part A. A Field Trip**

The destination may be any natural area commonly called "grassy meadow," "savannah," "evergreen forest," "deciduous forest," etc., or any combination of these and some others. On this trip note the kinds of plants in each "community" which falls into these and estimate roughly the percentage of each.

| CATEGORY | SYMBOL |
|---|---|
| Canopy Layer (at least 20 meters or 65 feet tall) | |
| Subcanopy Layer (not over 65 feet tall) | |
| Shrubs (1-3 meters tall) | |
| Herbs (non-woody plants, many of which are tall) | |
| Floor covering (may be leaves, needles, etc.) | |

| PLANT TYPES | SYMBOL |
|---|---|
| Woody Vegetation | |
| Deciduous | D |
| Evergreen | E |
| Herbaceous Vegetation | |
| Grass | G |
| Non-grass | N |




Reading from Left to Right: 1 Evergreen, under 65 ft.; 3 species of deciduous trees over 65 ft. with a non-grass floor cover; 3 species of deciduous and 2 species of evergreens under 65 ft. on the slope; 2 evergreen and 3 deciduous species of shrub are found beneath a canopy formed of a single deciduous species. Brook. Five species of grasses and 7 of non-grasses are found beneath a grove of tall (over 65 ft.) deciduous trees and 4 kinds of deciduous shrubs.

Your team will be assigned a strip about 10 to 20 yards wide extending through the community. Prepare a profile of the strip using the symbols listed above.

**Part B. Study of A Site Within a Community**

A plot will be laid out for the class and your team will be assigned a subplot to investigate. Plot sizes will probably be as follows:

Trees: 10 x 10 meters
Shrubs or medium-sized vegetation 2 x 2 meters
Low vegetation (herbs) 1 x 1 meter

Record the positions of various kinds of plants on a grid representing your subplot. Also indicate the location of animal burrows, observed birds, insects on the ground, vegetation and in the air, etc. Don't forget to turn over a few rocks to see what's underneath.

While surveying the various types of plants use these codes for density:

c = continuous growth
i = individual plants, usually not touching each other
p = patches plus woody plants scattered alone or in groves; herbs in patches
r = rare, but conspicuous
b = barren–vegetation largely or entirely missing.

Light is an important physical factor. Use a light meter to measure the amount of light on sunny areas, in shade beside shrubs and under trees. (Measure seconds exposure at f11 using a film speed of 25 (Kodachrome).)

## REPORT SHEET FOR EXERCISE 38

**Part A. A Field Trip**

Kind of Community _____

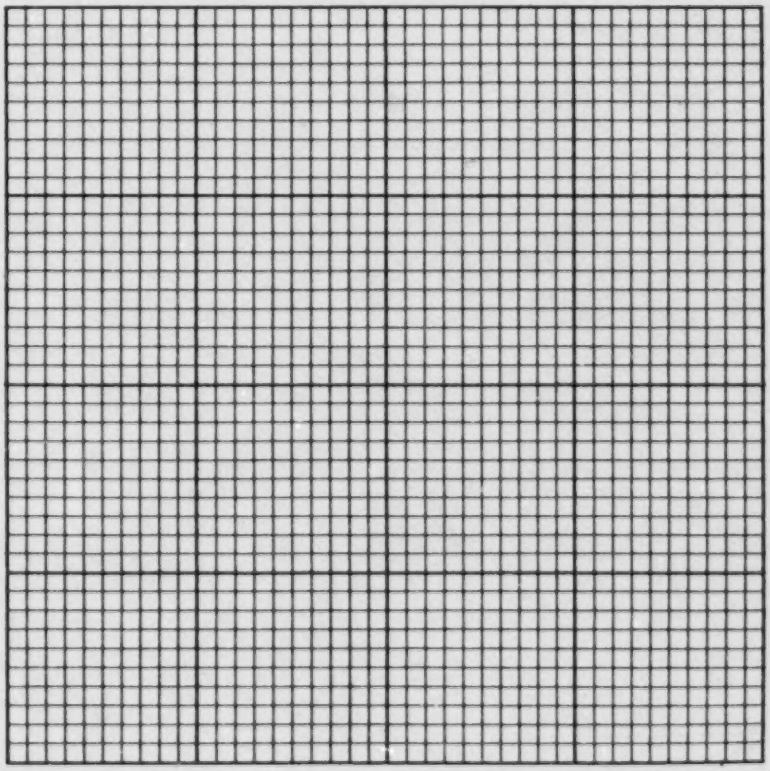
Location _____

Season _____

LAND ELEVATION

DISTANCE ALONG LINE OF PROFILE

**Part B. Study of a Site Within a Community**



Type of Community _____ Season _____

Location _____

# EXERCISE 39 – SUCCESSION FROM ONE ENVIRONMENT TO ANOTHER

*(A Field Exercise)*

In the study of development we distinguish between a modulation and differentiations. A modulation is a change that is reversible and temporary, like contraction and relaxation. Differentiation is a change which is not reversible, or at least not easily so. Some communities of plants, for example a meadow, may be subject to certain temporary changes such as the appearance of seasonal plants, dormancy due to a lack of water, being covered with a layer of mud by a flood, and so forth. These conditions are reversed with a return to "normal" conditions. Other environments, such as a pond and its shoreline represent two separate environments which grade into each other. Sometimes the zone of gradation is wide and at other times it is very narrow.

Materials and Equipment

Field notebook and pencil
1 foot ruler marked in cm.
Small boat
Wading boots
Weighted line equipped with hooks
Geotomes (shovels, mattocks)
Sieves
Plankton net
Glass-bottomed buckets or boxes for underwater observation

Procedure for Field Trip

You will be divided into teams. Each member will have a specific job to do, such as recorder, digger, dipper, siever, sample collector and carrier, etc. Your instructor may assign each group in the class a particular kind of environment to study and afterwards arrange for notes to be compared so that you will get the whole picture. An alternative is to assign you a strip traversing all of the environments in the grade and have you study the various organisms in the succession.

**Part A. Plant Succession Starting With Open Water**

We shall make observations on the kind of biological organisms in and around a pond, but particularly plants, because they will stay put longer so you can see them. The environments to be considered will be deep water, shallow water, exposed mud (wet soil), damp soil, and dry land (with deeper underground water) and perhaps high ground if there is any nearby.

When you arrive at the pond ask yourself if there is any apparent arrangement of the plant communities with regard to light and water. Is the pond bottom near the shore sandy and clean or covered with decaying plant material (sediment)? Is the water clean and clear or is it filled with the bloom of algae?

If there is only one boat, then only one team at a time will be sent to investigate the deeper waters of the pond. Wading boots provide more protection for waders than do sneakers, but in any event one should not investigate the shallows wading barefooted therein.

In deep waters look for animals such as fish, shrimp, and other aquatic creatures. Of course, collect and determine the types of submerged plants. Use the plankton net for collecting more minute members of the community. Drag the weighted hook along the bottom and see what you can bring up of biological interest. What similar characteristics do you see among the submerged plants?

In shallower water notice plants that are rooted in the bottom but have submerged leaves and also those that have both submerged and floating leaves. Is there a limit to how far out aquatics are found? How near to the shore? Use the observation bucket or box and gently push back the leaf cover. What kinds of animals do you see pass by beneath the leaves? Examine the underside of some leaves. Are there any snails or other animals attached?

At the edge of the water there will usually be a variety of bullrushes, grasses of other types including "cat tails," etc. Are there floating plants?

In wet sand-loam (mud) observe communities of sedge-grasses (swamp grass). Do you see any birds in this area? Are there any herbs or shrubs?

On dry and higher ground what kind of trees are to be found? Are they near the water or further away? If they are located back from the pond are there herbs and shrubs in between? Does the tree community overlap herbs down to the edge of the water? The final community is called the climax community. It may be either trees or grassland. Why should it be either of these?

Succession At An Abandoned Farmsite or Forest Cutting

Removal of the trees and underbrush during logging operations or the clearing of land for farming will bring the community picture back to herbs and grass. When these sites are abandoned then the succession begins back toward trees. If the class can visit such a site, determine the state of the site and predict what will happen in the next few years. This is more easily done if the time of the cutting or abandonment is known.

**Part B. Plant Succession Starting From Bare Rock Areas**

Outcroppings of rock of sufficient size will provide a group of concentric communities (compare with those around ponds). Determine whether these are based upon the availability of water or upon the depth of the soil or both.

*Plants on the Bare Rock.* Examine the bare rock for the presence of living material. The crust-like (crustose) plants here may be gray, green, black or yellowish-orange. What is the rock surface like beneath these plants? List the varieties.

*Shallow Soil in Cracks and Crevices.* Such soil is made up of grains of rock, plant litter and settled dust which forms a substrate which may be only a few millimeters deep for the rooting of plants. List the kinds you find here. Are they like the bare-rock plants in any way? Measure the depth of the soil. Can you give any reason why the plants in this shallow soil should crowd out the bare rock plants?

*Shallow Soil Over Rock Substrate.* List the kinds of plants to be found where the soil is only a few centimeters deep. Because of its shallowness it may not be able to hold much water and may be dry.

*Established Soil.* This area usually is inhabited by associations of herbaceous plants. List the kinds present. Is there a gradation through shrubs to trees or are these kinds of communities overlapping? Map the areas occupied by herbs, shrubs and trees. What kinds of plants form the climax community at the site you are studying?

Re-assemble promptly on call or signal from the instructor. Cap all liquids securely for transport. Start preservation procedures as soon as possible after return to the laboratory.

Report

Draw a map of the sites you have examined indicating the kinds of environments and the kinds of plants found within each plus any other data of interest such as birds or other animals associated with the site. Refer to Exercise 1 for the structure of a scientific report.

# EXERCISE 40 – EFFECT OF PHYSICAL AND CHEMICAL FACTORS ON ANIMALS

Materials and Equipment

pH paper
Petri dishes
HCl (diluted)
Sodium hydroxide (diluted)
Medicine droppers
Ehrlenmeyer flasks
Cotton
1 liter beaker

Living Materials

Ants
Paramecium
Rana pipiens
Daphnia
Cyclops
Various other animals may be used

Procedures

**Part A. The Effect of Temperature**

The effect of temperature is most easily demonstrated on poikilothermic animals such as amphibians or insects.

1. A frog may be used to demonstrate tolerance to a temperature range quite simply. Place a frog in a jar with one inch of water at about 25°C. The jar should have a perforated lid, and a thermometer should be inserted through one of the perforations so that the temperature of the water next to the frog may be recorded. Place this apparatus within an ice bath (a large beaker covered with crushed ice and some salt on its bottom will do). Every five minutes or less (according to how rapidly the temperature of the water changes) record the rate of buccal pumping (movement of the floor of the mouth) of the frog. This is equivalent to measuring how often we breathe per minute. Keep records until the temperature no longer drops. Observe and record the change in activity pattern of the frog.

Repeat this experiment, this time, with a hot water bath raising the temperature steadily until 50°C is reached. Repeat records of buccal pump rate and activity as above. Is there ever too much of a good thing?

2. Place one ant in the bottom of a 250cc ehrlenmeyer flask, plug the lid with cotton if necessary. Place the ant in water both at room temperature. Record the approximate distance he walks in a minute. (This can be estimated if you know the distance across the bottom of the erlenmeyer flask.) Now begin cooling the flask by addition of cold tap water–cool it, 5°C and again record the ant's activity, continue cooling and recording. Ice should be added when the tap water is no longer adequate. Record and cool until the temperature no longer decreases–record at several 5 minute intervals at the lowest temperature obtained.

Now, reverse the experiment, raising the temperature in 5°C units and recording distance traveled in a minute until a temperature of 90°C is reached.

NOTE: At each temperature allow a minute or two before measuring the ant's activity.

**Part B. The Effect of Hydrogen Ion Concentration (pH)**

All organisms depend on a tolerable range of hydrogen ion concentration. It is most easily demonstrated with small aquatic creatures.

1. The effects of pH on *Paramecium sp.* is easily demonstrated. Set up a series of petri dishes (standard size) so that each dish will have about 25cc of water (tap water will do as well as distilled water). Obtain a dilute solution of HCl and also one of NaOH. Add acid or base dropwise into the different dishes, measuring your effect with pH paper until you have a range of pH of perhaps 2 to 12. (It is not necessary to have exactly 12 dishes nor to have pH's of any exact value, the objective is to get the range and not make the intervals too large.)

Obtain a dense culture of *Paramecium.* Pipette 1 ml. of this culture into each of the dishes you have prepared. At 5 minute intervals for ½ hour record the percent alive and dead in each dish. This will require the use of a dissecting microscope.

2. This same situation can be used with cultures of any microcrustacean types available (e.g., *Daphnia, Cyclops,* or other small aquatic animals such as rotifers. If *Daphnia* or *Cyclops* is used, these are large enough so that a microscope will not be necessary. If species are available from acidic waters (e.g., a sphagnum bog) their tolerances may be compared with those obtained from waters approaching neutrality or perhaps being a little basic (10-20 animals/dish will be adequate; if possible replicates should always be set-up).

## REPORT SHEET FOR EXERCISE 40

### Effect of Physical and Chemical Factors on Animals

**Part A. The Effect of Temperature**

| ANIMAL | FEATURE BEING MEASURED | TEMPERATURE | RATE | | MEAN |
|---|---|---|---|---|---|
| | | | TRIAL 1 | TRIAL 2 | |
| | | | | | |

**Part B. The Effect of Hydrogen Ion Concentration (pH)**

| ANIMAL | DISH NUMBER | pH | NUMBER AFTER MIN. | | PER CENT ALIVE |
|---|---|---|---|---|---|
| | | | ALIVE | DEAD | |
| | | | | | |

## EXERCISE 41 – TERMITE-FLAGELLATE INTERACTION

Materials and Equipment

| | |
|---|---|
| Microscope slide | Forceps |
| Tap water or saline solution | Cover slip |
| Microscope | Live termite |

Procedure

Obtain a termite from the jar and place it on a slide. With a forcep or dissecting pin squeeze its abdomen until the contents are evacuated or the abdomen is squashed. Place a drop of tap water or a slightly saline solution (why is the latter better?) on the abdominal contents, push the rest of the termite aside, and place a cover slip over the material. Examine under the compound microscope.

Conclusion: Questions for Discussion

1. Are there living organisms present?

2. What do these organisms look like?

3. What is the possible relationship between the termite and these microorganisms?

4. How could you test the hypothesis that the relatinship is an obligatory mutualism?

5. What does a termite eat? Can you eat this? Can most animals eat this? What is the nutrient?

## REPORT SHEET FOR EXERCISE 41

**Termite-Flagellate Interaction**

Question 2. Above are some sketches of what the organisms looked like.

3. What is the possible relationship between the termite and these microorganisms?

4. How could you test the hypothesis that the relationship is an obligatory mutalism?

5. What does a termite eat? Can you eat this? Can you digest it? Can many animals eat this substance? How do animals get nutrients out of this substance?

# EXERCISE 42 – GROWTH OF A POPULATION[1]

*(Yeast Culture)*

Materials and Equipment

Yeast (e.g., like tinfoil packages sold in groceries)
Sugar
Pipettes (eye-dropper with narrow bore)
Microscopes
Distilled water
Stage micrometers or metric plastic rules
Test tubes
Microscope slides
Coverslips (microscope)

Procedure

1. Your teacher will prepare a 5 to 10% aqueous solution of sugar or molasses. Add one-half of a package of dried yeast to 500 ml. of medium. (Increase growth by adding 15 to 25 beans or peas.)

2. Place most (about 475 ml.) of the culture in fermentation tubes, leaving enough for the group to get an initial count of yeast cells per field (f). Place the tubes in a warm area (25 to 30°C – 77 to 86°F). Within 6 to 24 hours a sample of the culture should be investigated for possible changes.* At this point data collecting may begin by periodically checking the culture for a change in yeast population (every 6, 12, or 24 hours). This data collected is plotted on a graph.

Measurements

1. Determine the area of a coverslip (K)
   - Square . . . . . side squared
   - Circle . . . . . pi x radius squared

2. Determine the area of a microscope field
   - Low power microscope field (A) 100X
   - High dry microscope field (B) 440X

Hint

The diameter of the microscope field can be measured directly with the aid of a stage micrometer or a small clear plastic rule (metric).

*Would the lag phase be longer or shorter if the culture were placed in a refrigerator? What would be the effect on the time axis of the graph if we started the yeast with 1/20 package in 500 cc instead of ½ package?

[1]Courtesy Robert J. Anthony, Jackson State College.

3. Take a known volume (V) of a sample, place it on a slide with an eye dropper pipette. Gently place a cover slip over the sample (if liquid emerges beyond the edges of cover slip repeat the process until it does not occur). Count the number of cells observed in a microscopic field. Then take similar samples at intervals of 6, 12 or 24 hours and record your observations.

4. Let (f) equal the number of fields counted.

5. Let (N) equal to the number of organisms counted per field (counted organisms/field). If the number per field is larger than 150 organisms, dilute.

6. The value A is the area of the low power field if you use high power substitute B in the place of A. You have calculated both in two above.

7. (K/A) N (1 ml./V) equal to the number of organisms per millimeter (ml).

Hint

A good rule for sample counting is to obtain N for at least 10 fields or to count as many fields necessary to obtain an N of at least 100 if organisms are very sparse.

Calibration

Your teacher will supply you with eye dropper pipettes and some graduate cylinders (10 cc to 25 cc). Calibrate the pipette by counting and recording the number of drops of water needed to produce 5 cc of water. You are now able to determine the number of drops in 1 cc and the value of one drop of water of your sample taken under three above.

Sample Problem

If 100 drops equal to 5 cc (100/5 cc) then there are 20 drops in 1 cc (20/cc). Therefore, 1 drop is equal to 1/20 of a cc or 0.05 cc. This represents the value, V, in the equation. Suppose K = 400mm$^2$, and A = 5mm$^2$, and the total count for 10 fields, N, = 50 then by seven above.

$$\frac{400}{5}\ \frac{(50)1}{.05} = 80\ (50)\ 20 = 80{,}000/\text{ml}.$$

## REPORT SHEET FOR EXERCISE 42

Area of the microscope fields: Low Power:

High Power:

Number of cells per field on HP after the following times

| Time | Cells per Field |
|---|---|
| | |